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THE CHEMICAL TOXICITY OF URANIUM WITH SPECIAL REFERENCE TO EFFECTS ON THE KIDNEY AND THE USE OF URINE FOR BIOLOGICAL MONITORING

A research report prepared for the Atomic Energy Control Board by

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RESEARCH REPORT

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SUMMARY AND RECOMMENDATIONS

In the early days of the Manhattan Project a very extensive toxicological programme was mounted with one of its principal objectives the setting of exposure limits for airborne uranium in the workplace based upon uranium's known chemical damage to the kidney.

Those connected with the studies at that time proposed an airborne uranium exposure limit of 50 micrograms per cubic metre for soluble uranium compounds, and there is some evidence, based on the actual hours of exposure for the animals in the study, that this exposure was incorrectly calculated and was set too high.

The countries of Eastern Europe generally adhere to the exposure limits proposed by the Soviet Union which are even lower than the 50 micrograms per cubic metre level, being 15 micrograms for soluble uranium compounds, but there are no published documents which provide the scientific basis for the Soviet figures. Despite these low exposure limits, the opinion in the uranium industry has been that no adverse health effects have been seen in uranium workers exposed to the much higher exposure limit of 200 micrograms per cubic metre, or to the even higher levels which have been estimated to have been common in the early days of the industry. Unfortunately, no adequate epidemiological studies have been carried out to substantiate the opinion of those in the industry and so little data derived from human sources can be set against the considerable body of animal data. Faced with this situation regulatory agencies, who are being increasingly pressed to provide public, scientific documentation for their exposure limits, must rely very heavily on the data derived from animal studies.

Urinalysis as a means of biological monitoring has been common in the uranium industry, but this has usually meant monitoring urine uranium concentrations which is of limited value in detecting accidental cases of overexposure or in predicting the body burden of uranium. Urinary uranium levels used to provide group averages can be of value in showing trends in the absorption of uranium on a group basis, and such knowledge may be valuable from an occupational hygiene viewpoint. The other constituents and properties of the urine have generally been neglected. Protein, enzymes, B_2 microglobulins, amino acids, citrate and specific gravity (osmolality) are relatively easy measurements to make and could be used as indicators of renal function in epidemiological

studies. Such studies have only been carried out in animals and need to be verified as useful in human working populations. If no abnormal urinary constituents are found in persons exposed to uranium, there remains the problem of interpreting negative findings. In animals, most of the abnormal urinary findings may revert to normal with continued mild to moderate exposure, although the kidney may continue to show histological changes and abnormal citrate and amino acid excretion may persist. Whether such a "tolerant" kidney should be regarded as "normal" becomes important in considering the setting of exposure standards.

Little attention has been paid in recent years to the possible effects of uranium exposure on reproduction. At the time of the original toxicological studies during the Second World War, two studies were carried out, one of which was to high levels of uranium and the other only a brief 24-hour exposure, but both showed statistically significant effects on reproduction. The studies have not been repeated by other investigators and so questions remain to be answered as to the effect of uranium on reproduction.

Recommendations

1. There is a need for scientific documentation of the present airborne exposure limits for uranium workers. Such documentation could be sought through soundly planned epidemiological studies using:

   (a) A register of persons in uranium exposure with long-term mortality follow-up for all causes of death.

   (b) A cross-sectional study of various measures of renal function in uranium-exposed and non-exposed populations. (If possible, with persons having different levels of uranium exposure.)

   (c) A prospective comparison of the health of two cohorts of uranium-exposed and non-exposed individuals with particular emphasis on renal disorders.

(d) To study, in a selected group of persons entering uranium exposure for the first time, renal function to establish whether at realistic levels of exposure renal "tolerance" develops to uranium in man.

In addition to these epidemiological studies in human populations, some further animal studies (beyond those in progress) are indicated.

(e) A well-planned scientific study, in animals, of the reproductive effects of uranium to amplify the findings of the studies done during the Manhattan Project.

(f) At the time of the Manhattan Project, studies of the effect of chemicals on neurological and behavioural functioning were not routine and there has been little or no such testing in the case of uranium. Heavy metals characteristically affect the nervous system, e.g. lead, mercury, manganese, etc., and an adequate search for such effects due to uranium should be carried out.

When some or all of these studies are sufficiently far advanced for useful data to be available, a conference should be arranged to consider existing exposure limits for uranium in the light of both the human and animal data.

RÉSUMÉ ET RECOMMANDATIONS

Dès les premiers jours du projet Manhattan, un programme très complet d'études toxicologiques a été mis sur pied. Il visait surtout à établir des limites d'exposition à l'uranium en suspension dans l'air dans le milieu de travail, à la lumière de la détérioration chimique connue du rein sous l'effet de l'uranium.

Les responsables des études avaient, à l'époque, proposé une limite de 50 microgrammes par mètre cube pour les composés solubles d'uranium; or, il semblerait maintenant, selon le temps réel d'exposition des animaux pendant l'étude, que cette limite a été mal calculée et qu'elle est trop élevée.

Les pays d'Europe de l'Est souscrivent habituellement aux limites d'exposition encore plus faibles proposées par l'URSS, soit 15 microgrammes par mètre cube pour les composés solubles d'uranium, mais aucun document expliquant comment les Soviétiques en sont arrivés à ce chiffre n'est publié. En dépit de ces faibles limites, les membres de l'industrie sont généralement d'avis qu'aucune conséquence nocive sur la santé n'a été décelée chez les travailleurs de l'uranium exposés à des quantités plus élevées de 200 microgrammes par mètre cube, par exemple, et à des quantités même plus élevées, que l'on estime avoir été courantes au début de l'industrie. Malheureusement, aucune étude épidémiologique appropriée n'a été effectuée afin de justifier cette opinion, et il existe très peu de données sur l'homme qui peuvent être comparées aux données considérables des effets sur les animaux. C'est pourquoi les organismes de réglementation, que l'on presse de plus en plus de rendre publique de la documentation scientifique sur leurs limites d'exposition, doivent compter fortement sur les données tirées des études sur les animaux.

Les analyses d'urine pour surveiller les effets biologiques sont courantes dans l'industrie de l'uranium, mais ces analyses consistent habituellement à surveiller les concentrations d'uranium dans l'urine, ce qui est d'une valeur limitée pour détecter les cas accidentels de surexposition ou pour prédire l'effet de l'uranium sur le corps. Les concentrations d'uranium dans l'urine utilisées pour établir des moyennes de groupe peuvent être utiles pour montrer les tendances d'absorption de l'uranium dans un groupe, et ces connaissances peuvent être précieuses au plan de la sécurité professionnelle. Les autres éléments et propriétés de l'urine ont, dans l'ensemble, été négligés. Il est relativement facile de mesurer les protéines, les enzymes, les microglobulines de B_2, les acides aminés, les citrates et la densité spécifique (osmolalité) qui pourraient servir d'indicateurs du fonctionnement des reins dans les études épidémiologiques. Les études du genre n'ont été effectuées que sur des animaux, et il faut en vérifier l'utilité chez l'homme. Si aucun élément anormal de l'urine n'est décelé chez des personnes exposées à l'uranium, il reste à interpréter les résultats négatifs. Chez les animaux, la plupart des anomalies peuvent être redressées malgré une exposition faible ou moyenne, bien que le rein puisse continuer à présenter des changements histologiques et que des excrétions anormales de citrates et d'acides aminés puissent persister. Il devient alors important, lorsqu'on étudie l'établissement de normes d'exposition, de décider si un rein "tolérant" peut être considéré comme étant "normal".

Au cours des dernières années, on a porté très peu d'attention aux effets éventuels de l'exposition à l'uranium sur la fonction reproductive. Lors des études toxicologiques initiales effectuées pendant la Deuxième Guerre mondiale, deux études ont été menées, dont l'une portait sur des concentrations élevées d'uranium, et l'autre, uniquement sur une brève exposition de 24 heures; les deux prouvaient statistiquement que l'uranium a des effets considérables sur la reproduction. Les études n'ont pas été répétées par d'autres enquêteurs, de sorte que l'on s'interroge toujours sur l'effet de l'uranium sur cette fonction.

Recommandations

1. Il est nécessaire de réunir de la documentation scientifique sur les limites actuelles d'exposition des travailleurs de l'uranium aux particules en suspension dans l'air. Cette documentation pourrait être réunie au moyen d'études épidémiologiques bien planifiées, qui se fonderaient sur:

   (a) un dossier des personnes exposées à l'uranium, avec rappel à long terme sur les décès, quelle qu'en soit la cause;

   (b) une étude transversale des divers relevés de la fonction rénale chez des groupes de personnes exposées et non exposées à l'uranium (dans la mesure du possible, des personnes ayant été exposées à des concentrations différentes);

   (c) une comparaison éventuelle de l'état de santé de deux groupes de personnes exposées et non exposées à l'uranium, avec insistance sur les affections rénales;

   (d) l'étude, au sein d'un groupe choisi de personnes exposées à l'uranium pour la première fois, des fonctions rénales afin d'établir si l'homme peut développer une "tolérance" rénale à l'uranium lorsque les niveaux d'exposition sont réalistes.

   En plus de ces études épidémiologiques de personnes, quelques études supplémentaires sur des animaux (plus poussées que celles qui sont en cours) s'imposent;

   (e) une étude scientifique bien planifiée des effets de l'uranium sur la reproduction des animaux, afin de suppléer aux conclusions des études effectuées dans le cadre du projet Manhattan;

   (f) dans le cadre du projet Manhattan, l'étude des effets de produits chimiques sur les fonctions neurologiques et sur le comportement n'était pas courante et, dans le cas de l'uranium, très peu d'essais, sinon aucun, ont été effectués. Le système nerveux est particulièrement sensible aux métaux lourds comme le plomb, le mercure, le manganèse, et une recherche adéquate afin de déterminer si l'uranium a de tels effets devrait être effectuée.

Lorsque ces études, ou quelques-unes d'entre elles, seront suffisamment avancées pour produire des données utiles, une conférence devrait être organisée afin d'examiner les limites existantes d'exposition à l'uranium, à la lumière des données obtenues tant sur des humains que sur des animaux.

SECTION: 2.0

WORK STATEMENT

1. Based on a review of the literature (including ICRP-2, ICRP-6 and WASH 1251) discuss the use of kidney uranium levels as a basis for setting limits for human exposure to uranium.

2. Discuss the existing limits for the chemical toxicity of uranium in various countries (and recommendations for limits, in the cases of national or international bodies) as to both acute and chronic intakes below which no chemically toxic effects are expected to occur in humans. Comment on the suitability or justification of these limits.

3. In long-term biological monitoring of uranium workers:

   (a) Assess the usefulness of testing for protein (or other substances) in urine as an indicator of kidney damage.

   (b) Discuss the significance of levels of uranium in urine.

SECTION 3.0

CONCEPTS FOR SETTING HEALTH-BASED LIMITS FOR OCCUPATIONAL EXPOSURE TO URANIUM

A World Health Organization study group stated in 1980 that: "One major goal of occupational health programs is to prevent health impairment from exposure to harmful agents in the working environment."[1] An important tool in reaching this goal is to control the exposure of the worker to the harmful agents in such a manner that certain limits are not exceeded. The International Labour Organization adopted in 1977 the phrase "recommended health-based occupational exposure limit" to describe such control levels.[2]

The WHO study group[1] regard the setting of occupational exposure limits as a two step procedure. The first step is to define a level of exposure arrived at by experts that represents "no significant risk of adverse health effects" and which does not take into account technological and economic considerations. The second step is to convert these health-based limits into operational standards after discussion between the various interested parties.

In addition to health-based exposure limits for harmful agents in the external environment, the WHO study group support the concept of health-based biological limits. These are limits that represent levels of toxic substances or their metabolites in human biological material that will produce no adverse effect on human health. Although helpful in clarifying the roles of the various parties involved in the setting of occupational exposure limits, the Study Group on Early Detection of Health Impairment in Occupational Exposure to Health Hazards[3] recognized that the definition of "an adverse effect on health" contains an element of value judgment and will therefore differ to some degree from person to person. The U.S. National Academy of Sciences[4] has defined non-adverse health effects as:

(1) changes that occur with continued exposure and do not result in impairment of functional capacity or the ability to compensate for additional stress;

(2) changes that are reversible following cessation of exposure if such changes occur without detectable decrements in the ability of the organism to maintain homeostasis, and;

(3) changes that do not enhance the susceptibility of the organism to the deleterious effects of other environmental influences -- whether chemical, physical, microbiological, or social.

It is immediately obvious in reading these definitions that they are of only limited utility as the words "impairment," "decrement," and "deleterious" are used synonymously with adverse health effect and the definitions really only classify the conditions under which value judgments may have to be made. This point is illustrated later in this document in the discussion of the value of measuring enzyme levels in the urine of uranium workers.

The same criticism can be applied to the Academy of Science's listing of effects the committee felt were adverse:

" - effects that indicate early states of clinical disease;

- effects that are not readily reversible, and indicate a decrement in the body's ability to maintain homeostasis;

- effects that enhance the susceptibility of the individual to deleterious effects of other environmental influences;

- effects that cause relevant measurements to be outside the "normal" range, if they are considered as an early indication of decreased functional capacity; and

- effects that indicate important metabolic and biochemical changes."

For instance, with the wealth of information available on the biological effects of lead, there is still a spirited debate as to whether minor degrees of inhibition of the enzyme aminolevulinic acid dehydratase indicates an early stage of clinical disease[5] as a dose-related inhibition can be shown to occur at all levels of lead presently found in the human population.[6] Similar examples exist to show that each of the other adverse effects listed by the Academy of Sciences' Committee are subject to interpretation and therefore to discussion. One result of wrestling with the question of "When is a biological change an adverse health effect?" may be the conclusion that if this is not a purely scientific question, then perhaps non-scientists should be a part of this discussion process. This would mean that

even step one of the two step procedure outlined by the World Health Organization Study Group should not be looked upon as purely the domain of the scientist.

There can be little doubt, however, that step one requires the marshalling of all of the relevant scientific data and that this data must meet certain standards regarding its type and quality. The information required for health-based biological limits may be broadly divided into:

(1) The nature of the relationship between the measured biological index and the adverse effect on health against which the person is to be protected;

(2) The nature of the relationship between the measured biological index and the exposure of the individual to the harmful environmental agent; and

(3) The quality of analytical measurements involved. (Specificity, variance and accuracy.)

SECTION 4.0

THE NATURE OF THE RELATIONSHIP BETWEEN THE MEASURED BIOLOGICAL INDEX AND THE ADVERSE EFFECT ON HEALTH

The Critical Organ Concept

For measures to protect the health of uranium workers to be effective, there must be a broadly predictable relationship between the levels of the metal in the body and effects on health. Measures can then be aimed at preventing the uranium levels rising to the point where health effects might be expected. As not all tissues and organs will be damaged at the same level of uranium, it is a principle of occupational health that if the most sensitive organ can be protected, then the less sensitive organs and the person as a whole will also be protected. This principle has led the Scientific Committee on the Toxicology of Metals of the Permanent Commission and International Association on Occupational Health to formulate the concept of the "critical organ."

The lowest metal concentration at which adverse functional changes, reversible or irreversible, occur in the cell is known as the "critical organ concentration" and is defined as the mean concentration in an organ that would be necessary for a number of the most sensitive cells in the organ to be affected. It was pointed out that the critical concentration may differ considerably among individuals due to biological variations in sensitivity. The term "critical organ" was used to identify that particular organ which first attains the critical concentration of a metal under specified circumstances of exposure and for a given population.[8]

It is worth noting that the definition of the critical organ used in chemical toxicology is somewhat more "protective" of human health than the definition of the critical organ used in discussing the effects of ionising radiation in which the term refers to that organ of the body whose damage results in the greatest injury to the individual.

The Critical Organ in Uranium Exposure

In considering the chemical toxicology of uranium, the report of the Royal Commission of Inquiry into Uranium Mining in British Columbia[7] concludes that "the available experimental evidence supports the concept that the most susceptible organ system is the kidney." A number of more specifically toxicological texts have come to similar conclusions over the years. Thus, in the Handbook on the Toxicology of Metals, the chapter on Uranium states "the critical organ for the chemical toxicity (of uranium) is the proximal tubule of the kidney"[8] and the same conclusion is reached by reading two earlier but comprehensive textbooks on uranium toxicology.[9,10] The evidence from the literature is, therefore, unanimous that the kidney is the critical organ when considering the chemical toxicity of uranium. This does not, of course, mean that uranium has no harmful effects on other organs and organ systems in the body nor does it mean that subsequent information may not alter our views on the critical organ.

Exposure-Effect and Exposure-Response Concepts

The World Health Organization has defined these two terms.[1] An exposure-effect relationship is the relationship between quantified exposure and the quantitative severity of a health effect in an individual or group. An exposure-response relationship is the relationship between quantified exposure and the percentage of individuals with an effect of specified severity. It is important to distinguish between the two concepts and where possible utilize them both, as the exposure-effect concept is concerned with an <u>average</u> effect in all individuals at a given exposure level while the exposure-response relationship shows the individual variations in reaction at a given exposure level.

When using exposure-effect and exposure-response concepts, it is possible with decreasing levels of exposure to arrive at a level at which no adverse effect is seen or no adverse response is obtained. In practice, the number of observations upon which these levels are based is usually severely limited, and a more accurate phraseology would be "no detectable adverse effect level" or "no detectable adverse response level." Even these cautiously worded phrases must be used with care as the precision with which the levels are defined is heavily dependent upon the intervals between the exposure levels and the ability of the tests used to detect small changes in effect.

Reference has already been made to the problems associated with an adequate definition of an individual or group's exposure and this topic is so crucial and so often a weak link in developing exposure-response/effect relationship that it is worth further emphasis.

Inter-laboratory comparison (round robin test) results are essential in assessing a laboratory's ability to measure uranium in the working environment and more particularly in biological matrices. Such measurements should be reproducible within a standard deviation if much credence is to be given to the results. This subject will be touched on again.

Renal Damage Due to Uranium

There are many descriptions of the structural and functional disorders produced in the kidney by uranium. A composite description based on the best available information is found in reference 10 and is quoted here to provide a basis for later discussion.

The Results of Morphological Studies:

"The proximal convoluted tubules show the earliest change consisting of vacuolization and the accumulation of hyalin droplets and other cytoplasmic bodies. There is a lag period of from about 6 hours to several days in the appearance of these changes, depending on the dose, and ultimately, with administration of sufficient amounts of the material, cell necrosis is seen. The necrosis appears initially in the lower portion of the convoluted tubule. With lower doses, it is spotty in distribution and following higher doses, may extend as wedge-shaped areas into the outer cortex toward the capsule. Casts, either hyalin or containing shed necrotic cells, are present in considerable numbers after the first few days at all levels of the tubular system. After 2 or 3 days, evidence of regeneration of the lining of the convoluted tubules becomes apparent, depending, to some extent, on the severity of the initial injury. In non-fatal cases, after about 2 to 3 weeks, the lining epithelium is completely reformed but the individual cells are morphologically and functionally abnormal for considerably longer period of time. Regenerating epithelium takes several forms. Nuclei tend to be larger and darker

"than normal, with mitotic figures being frequently seen in the early stage. Cytoplasm is basophilic and often flattened. As regeneration continues, and the kidney becomes more and more normal in appearance, following sublethal doses of uranium compounds, subtle changes are seen for a considerable period of time, so that it is difficult to distinguish between regenerated cells and cells which have been "activated" in some fashion. The presence of calcium deposits within tubular epithelial cells and tubular casts has been described by many authors, while others have made no mention of such a change. Dahl (1953) carried out careful histochemical investigations of this process and concluded that, at least in the rat, it was the first change observed, as fine, specifically staining granules in the cytoplasm of proximal convoluted tubular cells with movement into luminal casts after cell rupture. The amount of calcium was dose-dependent. It is probable that larger intratubular deposits of calcium in casts are, in considerable measure, responsible for permanent blockage and the late changes of scarring and nephron atrophy described by many authors, particularly following continuous or repeated dosage.

"Reports of ultrastructural changes corresponding to the above described light-microscopic alterations have not been numerous but have helped to shed further light on the pathogenesis of the lesions. Mueller and Mason (1956) described changes at 6 hours in the mouse. About the only significant observation made by these authors was a progressive, swelling enlargement and disruption of mitochondria. They stated that basement membranes, cell borders, brush borders and background cytoplasm looked normal. Stone et al (1961) made electron microscopic observations between 1 and 6 days after injection of a lethal dose of uranium nitrate and observed marked swelling of the apical portion of the convoluted tubular epithelial cells with apical vacuoles and marked enlargement of sub-basilar compartments. These changes were maximal at the time polyuria was developing and were interpreted as being related to disturbed fluid transport. They appear to correspond with earlier descriptions of pallor and vacuolization of these cells as seen with the light microscope. Early pre-necrotic changes seen at one day, or later, consisted of swelling of mitochondria, endoplasmic

"reticulum and Golgi systems, as well as loss of microvilli of the brush border. Various cytoplasmic vacuoles and bodies, including myelin figures and hyalin droplets, increased in number, size and variety as proteinuria developed, and were thought to be suggestive evidence of tubular reabsorption of material from the tubular lumens.

"Porte et al (1963) have also described the early degenerative and necrotizing changes seen under the electron microscope in mice. Their findings were in essential agreement with those of Stone et al (1961), but they also included a study of regeneration, seen best at 6 days after administration of uranyl nitrate. At this time they observed viable cells scattered among the necrotic debris, and from these, thin cytoplasmic extensions were seen to progress along the basement membrane beneath necrotic cells. The regenerating cells were described as being "embryonic" in character, with large nucleoli, many free ribosomes, fine profiles of endoplasmic reticulum and a reduced number of mitochondria in which few cristae were seen. These cells showed no microvilli on their luminal surfaces and were joined together by "terminal bars" near their apices. There was frequent dilation of intracellular spaces. The findings of these authors would help to explain the microscopic appearance of those regenerating cells which have markedly basophilic cytoplasm and are flat, without evidence of brush borders.

"The preceding descriptions of the highly dramatic renal tubular changes resulting from uranium poisoning are undoubtedly of great importance, but have tended to overshadow the less marked and more subtle changes produced in the glomerulus. Despite the earlier reports of Christian and O'Hare (1913) and Susuki (1912), both of whom described moderate glomerular alterations in the kidneys of rabbits in addition to tubular changes, most authors who have discussed renal pathology in connection with uranium, have either denied glomerular changes or considered them insignificant. However, Hunter and Roberts (1932), using special stains, outlined glomerular changes in the kidneys of rabbits and monkeys, induced by uranium and other nephrotoxins which they interpreted as affecting largely the basement membranes of the glomerular capillaries, with development of some secondary fibrosis. Endothelial and epithelial degenerative and

"regenerative changes associated with formation of hyalin droplets were also noted. Finally, Benscome et al (1960) and Stone et al (1961) applied a variety of sophisticated special stains to 2-micron paraffin sections and confirmed their light microscope findings by electron microscopy and established the fact that striking glomerular changes did occur in rat kidneys damaged by uranium. A centrolobular lesion which developed after 3 days, contained fibres which stained like collagen and which subsequently were identified as such under the electron microscope, varying amounts of PAS-positive material were deposited in basement membranes adjacent to smaller lesions, there was vacuolization and hyalin droplet formation in epithelial cells and hyalin droplets and large globules of amorphous material were noted in the capsular spaces. The electron microscope revealed, in addition, a loss of foot processes, dense deposits between the basement membrane and the epithelium, as well as hyalin droplets, other cytoplasmic bodies, myelin figures and cytoplasmic vacuoles in epithelial cells. These changes are not unlike those seen in some spontaneous proteinuric states in humans and in some animals with experimentally induced proteinuria. It is possible, therefore, that some of the protein found in the urine following uranium administration or exposure, including plasma protein-bound uranium, may have escaped through the glomerulus. The nephron atrophy and scarring seen on occasions in the late stages of severe or repeated uranium poisoning, is probably related in some manner to the glomerular collagen deposition."

<u>The Results of Functional Studies</u>:

"Numerous studies of disturbed renal function, associated with uranium toxicity, have been reported. These have been directed at elucidation of the locus and mechanism of action of the metal on the kidney, the apparent tolerance which develops following repeated or continuous dosage, as well as in a search for a means of early detection, quantitation and prevention or treatment of the renal injury."

Renal Clearance Studies:

"As have been mentioned above, the clearance of uranium from the body is decreased by acidification of urine and increased by alkalinization with bicarbonate. The work of Walker et al (1941) and Pitts and Lotspeich (1946) indicated that the major site of reabsorption of bicarbonate is in the proximal convoluted tubule. This finding, in conjunction with the fact that 60 to 80% of water from the glomerular filtrate is reabsorbed in the convoluted segment, strongly suggests that the uranyl ion is available in this location for combination with lining cells of the tubules. Presumably the deleterious action results from binding or combination of the uranyl ion with the cell proteins, possibly in the surface membrane or after absorption through it in membranes of such organelles as mitochondria. Sodium bicarbonate has been among the successful materials used to prevent or treat acute uranium poisoning in experimental animals. It was most effective when administered parenterally and presumably acted by keeping the filterable uranium that passed through the glomerules in a complexed, and therefore a non-toxic, form. It is also known to increase the excretion of citrate in the urine and this citrate can also act to keep the uranium in a complexed state.

"Further evidence concerning the locus and, to some extent, the mechanism of uranium action on the kidney has been derived from studies of the renal clearance of various substances following uranium injection. Boby et al (1943) found all clearances decreased at the height of uranium injury in dogs, 5 days after subcutaneous injection of uranyl acetate. They interpreted the marked lowering of insulin clearance as due to back diffusion and of diodrast clearance as due to complete cessation of tubular secretion and pointed out that because of the severe nephron damage which occurs within a day or two after the administration of uranyl salts, only immediate effects on renal function would be valid in attempting to identify initial sites of uranium action. Consequently, renal clearances of 9 substances were measured within a period of about 2 hours after intravenous injection of uranyl acetate in rabbits. The findings were outlined by Dounce in chap. 15 of Voegtlin and Hodge (1949). The

"clearances of creatinine, insulin and xylose, which are considered to be measures of glomerular filtration, showed no significant change, indicating no early glomerular functional effect. Few significant changes were observed in the clearance of chloride and urea, both thought to be partially reabsorbed in the proximal convoluted tubular segment, and partially in more distal portions, of the nephron. Since it was considered likely that reduced reabsorption in some localized tubular region would be compensated for by increased reabsorption through some other portion, these clearances gave no information about the site of uranium action. Of the remaining substances studied, glucose and amino acids, which are normally reabsorbed in convoluted tubules, showed a significant increase in clearance, whereas the other two, phenol red and diodrast, believed to be secreted by epithelial cells into the proximal tubular lumen, showed a significant decrease in renal clearance. All the evidence derived from these clearance studies indicated that the major action of uranium is probably in the lower half or lower two-thirds of the proximal convoluted segment."

Proteinuria:

"An early and sensitive indication of uranium injury is the finding of protein in the urine. Of this protein, a sizeable fraction has been found, by electrophoresis, to be albumin. It is probable that a major part of the protein excreted in the urine results from failure of convoluted tubular epithelium to reabsorb the proteins which are found in very low concentrations in the large amount of glomerular filtrate which is normally concentrated about one hundred fold. Urinary protein is possibly enhanced to some degree by increased permeability of the glomerular membrane to plasma proteins, as suggested by the work of Stone et al (1961) and also by other proteins derived from damaged tubular cells, either by leakage from or by sloughing of these cells into the tubular lumens."

Enzyme Studies:

"Alkaline phosphatase was found by Breedis et al (1943) to be increased in the urine and decreased in kidney tubules when estimated histochemically. This suggested that other enzymes found in high concentration in the epithelium of kidney tubules might appear in the urine as a result of uranium injury. Studies which followed, reported by Dounce et al in chap. 14 of Voegtlin and Hodge (1949), demonstrated that the measurement of urinary catalase was one of the most sensitive early tests for uranium poisoning in some animals, particularly the rabbit. It was shown that very small doses of uranium, in the range of 0.01 to 0.02 mg/kg intravenously, increased the urinary catalase within a period of 2 to 4 days, and that the test was reproducible after repeated exposures. Fortunately, in tests on some humans exposed to uranium, no evidence of increased catalase excretion was found.

"Since alkaline phosphatase is known to be localized particularly in the brush border area of the proximal convoluted tubular epithelial cells, the appearance of this enzyme in the urine in uranium poisoning is further evidence of tubular damage in this segment. However, the tendency for it to be absent after further exposures and the finding of lower phosphatase activity in the kidneys of animals which had survived single or repeated doses of uranium, even after several months, indicated that newly regenerated cells in the proximal tubules were different from cells of the original undamaged tubule. In this respect, the studies of Wachstein (1957) on enzymatic staining reactions in regenerating tubular cells of rat kidneys following mercury poisoning, are of interest. Of 10 enzymes studied, all of which disappeared to a greater or lesser extent in the damaged kidney, there was a slow reappearance in the regenerating cells, in from 5 to 7 days after regeneration had begun, of the reaction for all enzymes except alkaline phosphatase. This enzyme, even after 28 days, could be detected only in trace amounts. The importance of alkaline phosphatase in uranium poisoning is also stressed by the findings of Albert et al (1963), who measured enzyme activities in kidney homogenates following intraperitoneal uranyl nitrate injection. Of 5 enzymes, the only change was noted in alkaline phosphatase, which fell to approximately 50% of the

"control value. This was observed at 24 hours after injection and continued throughout the course of the experiment which lasted 96 hours. While it is not known what role these changes in alkaline phosphatase played in the development and repair of renal tubular injuries, the findings appear to support the view that tolerance to repeated or continuous doses of uranium compounds is associated with biochemical as well as morphological alterations in the convoluted epithelial cell. While it has been established that uranium inactivated certain kidney enzymes, it is not known whether or not the immediate cause of the uranium damage to the kidney is enzyme blockage. Uranium is not a strong non-specific enzyme poison and much of the enzyme inhibition can probably be explained by the formation of uranium complexes with ionized acid groups of the protein component of the enzymes.

"The uranyl ion reversibly inhibits a number of enzyme systems such as citrate and hydroxyapatite. However, of 28 enzyme systems investigated in vitro, only 4 were susceptible to low concentrations of uranium. Certain metabolic reactions are also inhibited, such as those occurring on the cell surfaces of yeasts, the oxidation of glucose by the kidney and such reactions as oxidation of pyruvate, succinate and alanine. However, the amounts of uranium which inhibit these reactions are too small for testing for enzyme inhibition. The occurrence of a lag period from the administration of uranium to the appearance of renal damage, either functional or morphologic, is also more in favour of an indirect mechanism related to protein complexing in general than to a direct effect on enzymes, see chapter 13 of Voegtlin and Hodge (1949).

"On the basis of the studies outlined in this section, it is probable that the mechanism of the toxic action of uranium on the kidney cannot be explained in any simple fashion. All the following factors probably play some role in the final outcome; non-specific protein complexing, enzyme inhibition, interference with mitochondrial function, and membrane and fluid transport, as well as with the excretory functions of the tubular epithelial cells."

(For references contained in this Section, see Ref. 10.)

Exposure Effect/Response Levels for the Renal Effects of Uranium

Although a very large number of studies have been carried out in which various levels of uranium have been administered to a wider variety of animals, including man, very few of these studies provide data allowing dose-response relationships to be defined, using some form of kidney damage as the measured response. The picture has been further complicated by the intertwined concerns over damage due to ionising radiation and damage due to chemical toxicity. The units of measurement associated with these two classes of etiological agent are quite different, although under well-defined conditions, they may be related.*

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*For instance, the International Commission on Radiological Protection (ICRP) in 1958 defined the curie of natural uranium (referred to as the "special" curie) as corresponding to the sum of the following activities:

3.7×10^{10} disintegrations per second from Uranium-238

3.7×10^{10} disintegrations per second from Uranium-234 and

1.7×10^{9} disintegrations per second from Uranium-235.**

According to this definition, one curie of natural uranium corresponds to 7.57×10^{10} alpha disintegrations per second.

The specific activity of natural uranium is about 3×10^{6} g per special curie.[11] In units of mass, 7×10^{11} micro special curies per cubic metre is approximately equal to 200 micrograms of natural uranium per cubic metre of air and 3 g natural uranium corresponds to 1 micro special curie.

**The definition given here is correct. The contribution from U-235 was incorrectly given in the 1954 recommendation of the ICRP and ICRP Publication 2 (1959). The error was corrected in ICRP Publication 6 (1964), see ref. 9 of this report.

Results of Animal Studies

It is a continual source of frustration to those charged with the responsibility for suggesting exposure limits for chemicals based on toxicological data that the data available in the open literature so seldom provides exposure effect information. The majority of toxicological studies are concerned with documenting an effect but seldom with how that effect varies with the exposure level. This point is illustrated by the paucity of new data concerning uranium toxicity that can usefully be used in setting exposure limits. In fact, "the current standards for protection of workers in the uranium industry rest on a series of animal experiments performed for the Manhattan Project (1942-1945) at the University of Chicago and University of Rochester."[12] Although the experiments were carried out with the express purpose of providing information for setting exposure limits for workers in the Manhattan Project, the results did not allow a precise exposure-effect curve to be drawn. Indeed, at that time, it was customary to seek "no-effect" levels for the setting of standards and these levels were arrived at as a matter of judgment on the part of the investigators or regulatory bodies.

The conclusion of the toxicologists studying uranium toxicity in the Manhattan Project was that "as a guess 2-3 micrograms of uranium per gram might be deposited (in the kidney) without serious tissue damage." Ref. 9, page 2418. Although this statement is made in relationship to rat tissues, the same tissue concentration is used as a basic assumption by NCRP committee II[13] as set out in the following argument taken from ref. 9, page 252.

Assumption	Source
1) A maximum permissible concentration of 3 µg U per gram kidney	Animal experiment results (Voegtlin and Hodge, 1953; Committee judgment decision)
2) An average kidney mass of 300 g	Standard Man (ICRP Publication 2, 1959)
3) An effective half-life of 15 days for uranium in the kidney, i.e. 0.693/15 = 0.0462 x the kidney burden is excreted per day	Animal experiments: (Voegtlin and Hodge, Chap. 11, 1949). Experiments on man: (Struxness et al., 1955; Butterworth and McLean, 1955

Assumption	Source
4) That 2.8 percent of the uranium inhaled was deposited in the kidney (f_a as denoted by ICRP-NCRP)	The lung model (ICRP Publication 2, 1959) specifies that the 25% deposited in the pulmonary lung is absorbed into the body for soluble compounds. The 50% deposited in the upper respiratory tract is transferred to the gut and because of the negligible absorption of uranium can be neglected. Of the systemic uranium, 78% is rapidly excreted and the remaining 22% is divided equally between bone and kidney. $f_a = 0.25 \times 0.11 = 0.028$
5) That a worker breathes in an average of $6.9 \times 10^6 cm^3$ air per working day	Standard Man (ICRP Publication 2, 1959)

The calculation of the $(MPC)_a$ for soluble uranium then proceeds as follows:

1) The maximum permissible daily kidney input equals the daily rate of loss from the kidney when the burden is the maximum permissible = $0.0462 \times 900 = 41.5$ µg.

2) The corresponding lung daily input - $41.5/0.028 = 1480$ µg.

3) Therefore, the $(MPC)_a = 1480/6.9 \times 10^6 = 2.1 \times 10^{-4}$ µg/cm^3 = 210 g/m^3.

As listed in NCRP Subcommittee 2 Report (NBS Handbook 69, 1959), ICRP Publication 2 (1959) and ICRP Publication 6 (1962), this limit has been expressed in terms of µCi per cm^3 air using the special curie unit as used only for natural uranium and for natural thorium. Accordingly, 210 µg natural uranium per m^3 air converts to 7×10^{-11} µCi per cm^3 which is the value listed in the above references.

It will be quickly appreciated that in using both a mass concentration of uranium in air and a measure of radioactivity in a given volume of air, there is room for confusion as to which property of uranium was taken as the limiting factor.

A further possible source of confusion is the use of the term maximum permissible concentration (MPC) which has a different definition than the terms MAC (maximum allowable concentration) and TLV (threshold limit value) used in occupational hygiene practice for airborne contaminants. The MPC is the maximum permissible concentration of radionuclides in air which satisfies the requirement that the dose rate to the critical organ after 50 years of occupational exposure should not exceed the maximum permissible dose rates as set out by such bodies as ICRP and NCRP. The MPC is calculated based on the anatomical and physiological characteristics of the standard man.[14] However, the MPC for soluble uranium compounds is based on their chemical toxicity[15] which means that the calculations are the same as for a radiation based MPC but the controlling factor in the calculation is the maximum permissible concentration of uranium in the kidney (3 micrograms per gram) rather than the accumulated level of ionising radiation.

The calculation for setting a maximum intake of uranium in water $(MPC)_w$ drunk while at work is approached in the same manner as that for air and, again, this is set out in ref. 10.

The Derivation of the $(MPC)_w$ for Natural Uranium

The calculation for this occupationally less important limit proceeds in exactly the same manner as for the air limit but f_w, the fraction of the uranium taken by mouth which goes to the kidney is substituted for f_a and the average daily intake of water is substituted for the average daily intake of air. The values of these constants are as follows:

Assumption	Source
1) The fraction of the uranium taken in water which goes to the kidney is 0.0011	From oral intake experiments on man (see Section 4) a value of 1% absorption from gastrointestinal tract was selected 0.01 x 0.11 (see source material for air limit above) = 0.0011
2) The average daily intake (occupational) of water is 750 cm^3	Standard Man (ICRP Publication 2)

Therefore, if the average daily input to the kidney is set at 41.5 µg, the corresponding amount taken in by mouth is equal to 46 mg or 61 µg per cm^3 water. Employing the natural uranium curie for conversion gives a limit of 2×10^{-5} µCi per cm^3 water as listed in ICRP Publication 6 (1962).

Again, confusion is introduced into the calculation by converting the mass measurement of uranium in water into a measure of radioactivity in water. To a person familiar with the derivation of the units, the possibility of a serious error of judgment may be small but to a person without this background and dealing perhaps with more than one source of radioactivity in the drinking water, a needless error could be introduced. It is the uranium content of the water which is important in this situation and not the radioactivity derived from natural uranium.

Even further confusion is added to the selection of a "permissible level of uranium in the kidney" when Spoor and Hursch in ref. 9 comment on the derivation of the 3 microgram per gram figure used by ICRP.

"Comment: Basis for Selection of
3 µg per Gram Kidney as Permissible

It should be noted that this value is not listed as such in the ICRP tables but is derived from three other listed values; namely, "q" or permissible whole body content listed as 5×10^{-3} µCi, f_2 for kidney or the fraction of q, listed as 0.0065, which is contained in the kidney and 300 g which is listed as the kidney mass for the Standard Man. Thus,

$$\frac{5 \times 10^{-3}}{0.33 \times 10^{-4}} \times \frac{0.065}{300} = 3.2 \text{ µg/g.}"$$

It is interesting that this calculation based on the concern for the whole body content of radionuclides results in a figure not significantly different from the 2-3 microgram figure previously quoted from ref. 9.

In the tables that follow an attempt has been made to bring together the results of the Rochester and Chicago studies and, as far as is possible, to relate the pathological effects in the kidney to the tissue concentration of uranium.

SUMMARY OF INHALATION STUDIES (SOLUBLE URANIUM DUSTS)

(RENAL EFFECTS)

TABLE 1

COMPOUND	DUST CONCENTRATION (mg U/cu M)	DURATION	REMARKS		KIDNEY LEVEL (µg/g)
$UO_2(NO_3)_2.6H_2O$	9.8	30 days	Severe renal injury (gross only)	Dog Rabbit G.P.	0-4 0-12 0-3
$UO_2(NO_3)_2.6H_2O$	2.1	30 days	Renal injury & regeneration in dogs, slight to no changes in rats, severe renal damage in rabbits	Dog Rat Rabbits	0.4-4.3 0-100 0-14
$UO_2(NO_3)_2.6H_2O$	2.0	1 year	Mild to moderate renal tubular change in most animals	Dog Rat Rabbit G.P.	1.7 5.6 1.4 0.5
Animals exposed to $UO_2(NO_3)_2.6H_2O$ for 1 year after previous year of exposure to various soluble and insoluble dusts	2.0	2 years	Moderate renal tubular atrophy terminally	Dog Rat	0.2-12.3 0.3-5.8
$UO_2(NO_3)_2.6H_2O$	0.24	30 days	Rat: slight renal injury Rabbit: 8:20 severe renal injury	Rat Rabbit	0.0 0.0
$UO_2(NO_3)_2.6H_2O$	0.15	1 year	No effect	Dog Rat	0.5 1.4
$UO_2(NO_3)_2.6H_2O$	0.08	1 year	No effect	Dog Rat	0.4 0.4

SUMMARY OF INHALATION STUDIES (SOLUBLE URANIUM DUSTS)

(RENAL EFFECTS)

TABLE 2

COMPOUND	DUST CONCENTRATION (mg U/cu M)	DURATION	REMARKS		KIDNEY LEVEL (µg/g)
UF_6	13.3	30 days	Severe kidney damage	Dog Rat Rabbit	1.6-3.6 0.2-7 0-20
UF_6	2	30 days	Mild renal injury	Dog Rat Rabbit	0.4 2.7 0.3
UF_6	0.2	1 year	Mild renal injury with atrophic change	Dog	
UF_6	0.05	1 year	Extremely mild renal tubular changes in 1:13 dogs and 1:141 rats terminally	Dog	.35 µg/g
UO_2F_2	9.2	30 days	Severe renal damage	Dog Rat Rabbit Cat	10-34 0-60 0-15 9
UO_2F_2	1.9	30 days	Slight to moderate injury	Dog Rat Rabbit	1.2 3.3 4.7
UO_2F_2	0.46	30 days	No renal injury in rats, slight renal injury in rabbits		
UO_2F_2	0.15	30 days	Very slight renal injury in some dogs, 1 of 10 rats and 8 of 20 rabbits	Dog Rat Rabbit	0.2-0.3 0.2-2 <0.2

SUMMARY OF INHALATION STUDIES (SOLUBLE URANIUM DUSTS)

(RENAL EFFECTS)

TABLE 3

COMPOUND	DUST CONCENTRATION (mg U/cu M)	DURATION	REMARKS		KIDNEY LEVEL (µg/g)
UCl_4	11 mg	30 days	Moderately severe renal injury	Rat Rabbit G.P.	0-170 0-26 2-56
UCl_4	2.1	30 days	Mild to moderate renal injury	Rat G.P.	0-8 0-0.8
UCl_4	1.1	30 days	Moderate renal damage in dogs and rabbits but not in rats	NOT GIVEN	
UCl_4	0.25	30 days	Very mild renal changes	Rat Rabbit	0-7 0-13
UCl_4	0.2	1 year	Trace to moderate changes in some but not all animals terminally	Dog Rat Rabbit G.P.	0.2 0.4 0.4 0.1
UCl_4	0.05	1 year	Mild renal damage in 2:16 dogs, 6:50 rats	Dog Rat	0.2 0.4

SUMMARY OF INHALATION STUDIES (SOLUBLE URANIUM DUSTS)

(RENAL EFFECTS)

TABLE 4

COMPOUND	CONCENTRATION (mg U/cm M)	DURATION	REMARKS		KIDNEY LEVEL (µg/g)
UO_2	80 0.5 micron particles	30 days	Mild renal changes in rats Slight to moderate changes in rabbits	Rat Rabbit	3.0 1.2
UO_2	80 particle size > 1.0 micron	30 days	No renal injury in rats; slight to moderate changes in rabbits	Rat Rabbit	3.2 2.6
UO_2	10	1 year	Slight changes in dogs, very slight in rats or guinea pigs	Dog Rat Rabbit	1.7 1.1 0.5
UO_2	1	1 year	No evidence of renal injury	Dog Rat	0.7 0.4
U_3O_8	14	30 days	Evidence of kidney repair in 1 rat and 2 G.P.'s. Rabbits and mice normal	NOT GIVEN	
UF_4	14	30 days	Fairly uniform mild to moderate injury	NOT GIVEN	
UF_4	2.9	30 days	Very slight renal tubular changes	Rat	0.1
UF_4	0.5	1 year	No renal changes in dogs; borderline changes in 4 of 105 rats	Rat	0.3

(Continued)

SUMMARY OF INHALATION STUDIES (INSOLUBLE URANIUM DUSTS)

(RENAL EFFECTS)

TABLE 4 (Continued)

COMPOUND	DUST CONCENTRATION	DURATION	REMARKS		KIDNEY LEVEL
ORE	22	30 days	Moderate to moderately severe changes in kidneys	Rat Rabbit G.P.	4.3 1.7 9.9
ORE	3	30 days	Mild to moderate injury	Rat	3.9
ORE	0.6	30 days	Slight to no injury	Rat	3.8

In the tables that follow, the information contained in ref. 9 has been further condensed in an attempt to relate the kidney levels of uranium as closely as possible to the histological picture. While the results cannot be used to provide a graph of kidney uranium concentration versus the morphological damage caused by the uranium, it is possible to examine the validity of the 3 microgram per gram level as a possible benchmark for standard setting.

SUMMARY OF ORAL TOXICOLOGY STUDIES IN RATS

(RENAL EFFECTS)

TABLE 5

COMPOUND	CONCENTRATION IN DIET (AS COMPOUND)	NUMBER OF ANIMALS	DURATION	REMARKS	KIDNEY LEVEL (μgU/g)
UF_4	0.5%	6 male	2 years	No changes	0.2
UF_4	2.0%	6 male	2 years	No changes	0.5
UF_4	2.0%	5 female	2 years	No changes	0.5
UF_4	20.0%	7 male	2 years	Mild tubular degeneration	2.1
UF_4	20.0%	7 female	2 years	Mild tubular degeneration	11.8
UO_2F_2	0.01%	1 male	2 years	No changes	0.0
UO_2F_2	0.01%	14 female	2 years	No changes	0.53
UO_2F_2	0.05%	11 male	2 years	No changes	3.2
UO_2F_2	0.05%	6 female	2 years	No changes	1.1
UO_2F_2	0.10%	4 male	2 years	No changes	4.2
UO_2F_2	0.15%	5 male	2 years	Traces of tubular changes	5.6
UO_2F_2	0.25%	15 male	2 years	Moderate changes	10.9
UO_2F_2		9 female	2 years	Moderate changes	7.0
UO_2F_2	0.50%	2 male	2 years	Moderately severe renal damage	18.2
$UO_2(NO_3)_2.6H_2O$	0.5%	2 male(?)	2 years	Borderline changes	8.6*
	0.5%	5 female	2 years	Borderline changes	1.7*
	2.0%	5 male	2 years	Moderately severe	18.8*
	2.0%	5 female	2 years	Moderately severe	30.6*

*Levels reported at 1 year. No level given at time of sacrifice.

SUMMARY OF DATA ON 1 YEAR INHALATION EXPOSURE TO UO_2

TABLE 6

EXPOSURE LEVEL	RENAL URANIUM LEVEL (DOGS)	RENAL URANIUM LEVEL (RATS)
10 mgU/M^3	1.7 µgU/g	1.1 µgU/g
1 mgU/M^3	0.7 µgU/g	0.4 µgU/g

"Minimal renal pathological changes compatible with the effects of uranium poisoning were seen in 7 of 17 dogs exposed at the 10 mg level . . . no signs of uranium poisoning were seen in 124 rats and 14 guinea pigs at the 10 mg level or in 20 dogs at the 1 mg level."[9]

SUMMARY OF DATA ON 1 YEAR INHALATION EXPOSURE TO U Cl_4

TABLE 7

EXPOSURE LEVEL	RENAL URANIUM LEVEL (DOGS)	RENAL URANIUM LEVEL (RATS)
0.2 mg U/M^3	0.2 µgU/g	0.4 µgU/g
0.05 mg U/M^3	0.2 µgU/g	0.4 µgU/g

"The chronic inhalation of uranium tetrachloride dust at a concentration of 0.2 mg U/M^3 was of borderline toxicity for dogs and of decreasingly lower toxicity for rabbits, guinea pigs, and rats in the order named."[9]

Renal damage related to uranium exposure was seen in 50% of all dogs at the 0.2 mg level but only in 10% of dogs at the 0.05 mg U/M^3. The figures for rats were 9 and 6 percent respectively.

SUMMARY OF DATA ON 1 YEAR INHALATION EXPOSURE TO UF_6

TABLE 8

EXPOSURE LEVEL	RENAL URANIUM LEVEL (DOGS)	RENAL URANIUM LEVEL (RATS)
0.2 mg U/M^3	0.4 µgU/g	2.7 µgU/g
0.05 mg U/M^3	0.3 µgU/g	0.9 µgU/g

"Uranium hexafluoride inhaled by animals for a period of one year produced borderline toxicity at a concentration of 0.2 mg U/M^3 and only minimal effects at 0.05 mg U/M^3."[9] Twelve of 14 dogs showed mild renal-tubular injury at 0.2 mg U/M^3 while 2 of 17 dogs showed extremely mild renal-tubular injury at an exposure concentration of 0.05 mg U/M^3. The rest showed no evidence of morphological change due to Uranium.[9]

SUMMARY OF DATA ON 1 YEAR INHALATION EXPOSURE TO UF_4

TABLE 9

EXPOSURE LEVEL	RENAL URANIUM LEVEL (DOGS)	RENAL URANIUM LEVEL (RATS)
3 mg U/M^3	1.1 µgU/g	2.0 µgU/g
0.5 mg U/M^3	0.5 µgU/g	0.5 µgU/g

One year of exposure to 3 mg of U/M^3 resulted in definite but mild signs of toxicity in some animals of each of four species, dogs, rabbits, guinea pigs and rats. One year of exposure to 0.5 mg of U/M^3 resulted in no observable toxicity signs in dogs and barely detectable signs of toxicity in only 4 of 105 rats.[9]

SUMMARY OF DATA ON 2 YEAR

INHALATION EXPOSURE TO $UO_2(NO_3)_2 \cdot 6H_2O$

TABLE 10

EXPOSURE LEVEL	RENAL URANIUM LEVEL (DOGS)	RENAL URANIUM LEVEL (RATS)
2 mg U/M^3 (23 months)	2 µgU/g	0.4 µgU/g

One hundred percent of dogs (11 of 11) showed moderate tubular atrophy, 54% of rats showed mild tubular atrophy, (6 of 11). This 2 year study was conducted in 2 phases. During the first year the animals were exposed to various compounds and concentrations of uranium. During the second year the animals were exposed to 2 mg U/M^3 as Uranyl Nitrate. There appeared to be no relationship between the level of the previous exposure and the toxicity shown at the end of the second year. It was believed that the total exposure was probably responsible for the deaths of one rat and two dogs.[9]

FINDINGS IN DOGS AFTER 1 YEAR

INHALATION STUDY WITH URANYL NITRATE

PERCENTAGE OF ANIMALS SHOWING MORTALITY OR RENAL EFFECTS

TABLE 11

EXPOSURE LEVEL	ANIMALS DYING OF URANIUM POISONING	ANIMALS SHOWING RENAL INJURY	KIDNEY TISSUE LEVEL OF URANIUM (µgU/g)
2.0 mg U/M^3	4.2% (1:24)	93.8% (15:16)	1.7
0.25 mg U/M^3	4.0% (1:25)	84.0% (21:35)	1.0
0.15 mg U/M^3	0.0% (0:21)	0.0% (0:18)	0.5
0.04 mg U/M^3	0.0% (0:21)	0.0% (0:21)	0.4

FINDINGS IN RATS AFTER 1 YEAR

INHALATION STUDY WITH URANYL NITRATE

PERCENTAGE OF ANIMALS SHOWING MORTALITY OR RENAL EFFECTS

TABLE 12

EXPOSURE LEVEL	ANIMALS DYING OF URANIUM POISONING	ANIMALS SHOWING RENAL INJURY	KIDNEY TISSUE LEVEL OF URANIUM (µgU/g)
2.0 mg U/M^3	0.0 (0:203)	64.9 (100:154)	5.6
0.25 mg U/M^3	0.0 (0:194)	0.0 (0:10)	1.6
0.15 mg U/M^3	0.0 (0:171)	1.5 (2:135)	1.4
0.04 mg U/M^3	0.0 (0:173)	0.7 (1:140)	0.4

CORRELATION BETWEEN SUGGESTED SAFE LEVELS FROM REFERENCE 9 and RENAL LEVELS OF URANIUM

TABLE 13

DUST	URANIUM IN AIR (1 YEAR EXPOSURE) mg/M^3			
	NON-INJURIOUS OR SAFE	GENERALLY SAFE (OCCASIONAL SLIGHT INJURY)	LEVEL OF BORDERLINE INJURY	TOXIC LEVEL
UF_6		0.05(0.3)(0.9)*	0.2 (0.4)(2.7)*	
$U\ Cl_4$		0.05(0.2)(0.4)*	0.2 (0.2)(0.4)*	
$UO_2(NO_3)_2.6H_2O$	0.04(0.4)(0.4)*		0.25(1.0)(1.6)*	2.0(1.7)(5.6)*
$UO_2(NO_3)_2.6H_2O$	0.15(0.5)(1.4)*			
UF_4	0.5 (0.5)(0.5)*		3.0 (1.1)(2.0)*	
UO_2	1.0 (0.7)(0.4)*	10.0 (1.7)(1.1)*		

NOTE: Figures in parentheses are renal levels of uranium in micrograms of uranium per gram of wet tissue, levels for dogs without asterisk, levels for rats with asterisk.

"The concentration levels of the insoluble dusts which are safe and allowable are of a different order of magnitude from those of the soluble dusts; 10 mg U/M_3 as UO_2 is a permissible concentration for one year of daily exposure for even the most sensitive species, the dog, but 1 mg U/M^3 of this dust is absolutely without effect. On the other hand, 0.2 mg U/M^3 as one of the soluble compounds UF_6, $U\ Cl_4$ or Uranyl Nitrate Hexahydrate is not permissible for continued exposure, and it is seen that the levels must be lowered to 50 µg of Uranium per cubic meter of air for these compounds before exposure approaches a non-injurious level."[9]

Renal Functional Changes in Animals

In parallel with the morphological changes described in the last section, interference with renal function takes place and the severity of this interference is roughly proportional to the kidney concentration of uranium.[9] The impairment of renal function is not specific to uranium and results primarily from damage to the proximal convoluted tubule with lesser and more subtle changes in the glomerulus.[20,21,22]

An early indication of renal injury due to uranium is the appearance of a variety of enzymes in the urine of which catalase is considered the most sensitive indicator of renal damage. The catalase is thought to be released into the lumen of the nephron by the damaged tubular epithelial cells. It does not appear to be due to leakage of plasma catalase through the glomerulus. This conclusion is based on the higher molecular weight of plasma catalase and the fact that this is not found in the urine of animals severely poisoned with uranium.[9 (pages 889-950)] Associated with the early degenerative changes in the renal tubule and the release of catalase is the appearance of proteinuria and amino-aciduria. In the studies associated with the Manhattan Project, it was found that proteinuria and catalasuria were highly correlated in animals. Both tests tend to become positive in the same animals at the same time with a marked peak 3 to 5 days after the start of exposure, followed after 2 to 3 weeks by a return to near normal values. These changes in the urine follow the histopathological changes which show degenerative changes taking place in the proximal convoluted tubules followed by necrosis and sloughing of the dead cells into the lumen for about 7 to 10 days. Regeneration begins a few days after tubular damage occurs and this process is complete in about 2 to 3 weeks. Amino-aciduria, while appearing at about the same time as the proteinuria and catalasuria, remains elevated even after the repair process in the tubules is complete. The regenerated kidney, although apparently normal, cannot resorb amino acids as well as before the exposure to uranium. The concentration of amino acids in the urine varies considerably in isolated samples but is fairly constant in 24 hour collections. Correction for variations in the specific gravity of the urine reduces the variability in the measurement of amino-aciduria and it has been suggested that the amino-acid/creatinine ratio is a useful index of uranium poisoning, at least in rabbits.[9]

Exposure/Response Relationships for Amino-aciduria

In a study of 10 young rabbits given 0.3 mg/kg of uranyl nitrate hexahydrate, the aminoacid/creatinine ratio showed a sharp peak and then returned to normal. In an attempt to find the minimal level of uranium that would produce a detectable response in the aminoacid/creatinine ratio, the mean plus two standard deviations of the ratio in control animals unexposed to uranium was taken as the maximum level to be considered normal. This level was 10.7. The results are shown in the following table.

TABLE 14

AMINOACIDURIA IN RABBITS

Dose Mg U/Kg Body Weight	Number of Values Exceeding 10.7*
0 (control)	1/84
0.01	4/46
0.02	22/87
0.04	31/81

*Aminoacid/creatinine ratio

While this is the pattern in rabbits given a single exposure to uranium, when a group of young rabbits were given repeated doses, three different patterns of response emerged. With small doses, the peaks in the aminoacid/creatinine (A/C) ratio were similar after each dose, but the base line became slightly elevated. With doses of intermediate size, the initial peaks were higher than the subsequent peaks suggesting a degree of "tolerance" had developed in the animals. After large doses the peaks gradually increased and the base line rose to a higher level. In studies of rats exposed to uranium by inhalation proteinuria, catalasuria as well as the A/C ratio were measured. The results are shown in the following table.

TABLE 15

BIOCHEMICAL RESPONSE OF RATS TO THREE CONCENTRATIONS OF UO_2F_2

Dust Concentration* UO_2F_2 (Mg/M^3)	Number of Urine Values Above Normal Limits		
	A/C Ratio	Protein	Catalase
2.8	10:10	10:10	-
0.6	8:10	4:10	6:10
0.2	1:15	1:15	1:15

*30 Day Inhalation Exposure

The A/C ratio and catalasuria appear to be slightly more sensitive than proteinuria. An important difference between these two sets of observations is that while catalasuria and proteinuria return to normal at the end of 2 to 3 weeks, the A/C ratio remains elevated. It therefore appears that the mechanism leading to the elevation of the A/C ratio is different from that leading to catalasuria and proteinuria. (Ref. 9, pp. 2387-2391.)

That the biochemical variables do not necessarily bear a close relationship to the reported tissue levels of uranium is shown in the following table modified from Hodge et al. (Ref. 9.)

TABLE 16

Biochemical Variable	Max Normal	Control Value	UF_6 **		UCP_4		$UO_2(NO_3)_2.6H_2O$				UF_4		UO_2	
			0.2	0.05	0.2	0.05	2.0	0.25	0.15	0.04	3.0	0.5	10	1
BLOOD	mg%													
NPN	50	2.5	3.6	6.9	1.9	3.1	9.9	14	6.9	6.3	3.7	1.7	2.8	5.3
UREA N.	18	6											5.7	5.3
URINE														
PROTEIN	10	3.2	4.6	9.4	4.4	13.5	13.9	9.6	5.3	5.6	2.9	2.1	3.1	2.7
AAN/C*	1.9*	2.9	5.5	1.5										
RENAL TISSUE LEVEL OF U.		0.0	0.4	0.3	0.2	0.0	1.7	1.0	0.5	0.4	1.1	0.5	2.0	0.4

BIOCHEMICAL MEASUREMENTS OF CHRONIC INJURY IN DOGS

AT END OF ONE YEAR OF INHALATION OF URANIUM

(% of readings above maximum normal limit relative to total number of readings)

* Numerical ratio, amino-acid nitrogen to creatinine.

** Concentration of compounds expressed in mg U/M^3.

"Tolerance" to Uranium in Animals

As was mentioned in the preceding section on functional changes, the phenomenon of a reduction in the level of response after repeated doses of uranium compared with the level of response of the animals to the same dose given only once has been investigated by a number of research workers. Tolerance is defined as a lowering of the level of response of an animal to a lethal or severely damaging dose of a chemical after one or a series of smaller doses have been administered. Tolerance can be demonstrated with a variety of chemicals, OZONE, ANTU, LEAD, NICKEL and CADMIUM, but the occurrence of the phenomenon with many different chemicals does not imply a common mechanism.

Tolerance to uranium has been shown to occur in rats,[9] rabbits[23] and dogs[24] by a variety of routes of administration following two different dosage schedules. In one schedule a series of equal doses of a uranium compound is given followed by a dose calculated to be lethal. The second schedule consists of a series of doses starting well below the lethal level and then increasing with each administration until the last dose is at or above the calculated lethal level. Two mechanisms have been advanced for the production of tolerance to uranium. Haven (quoted in ref. 9, page 1900) working with rats found an increased excretion of citric acid after treatment of the animals with uranium. As citric acid can complex uranium, it was considered that one explanation of tolerance might be the complexing of a greater proportion of the uranium passing through the kidney and therefore effectively lowering the dose to the tubular cells.

The study by MacNider[24] suggested another explanation for the phenomenon of tolerance although the two mechanisms are not mutually exclusive. In this investigation, forty-one dogs were given two doses of uranyl nitrate hexahydrate. The first dose was 2 mg/kg while after an interval of two and a half to four weeks some dogs received a second injection of 2 mg/kg while some received a higher dose of 4 mg/kg. Eight of the forty-one dogs died as a result of treatment. By means of autopsy material and renal biopsies, it was shown that those animals which survived the second dose of uranyl nitrate had regenerated an atypical flattened epithelium in the proximal convoluted tubule while those that succumbed had regenerated a normal tubular epithelium.

A smaller study was carried out by Gruschow in which both the citrate excretion and renal histology were followed in seven dogs [9] (pages 1899-1905). An attempt to induce tolerance was made by both the "repeated doses" method and by the "increasing doses" technique. It was shown that both worked as well in dogs as they did in rats and that both mechanisms of tolerance appeared to also operate in dogs. The data from this investigation is set out in the following table.

TABLE 17

STUDY OF URANIUM TOLERANCE IN DOGS

Adapted from Ref. 9

Dog	Dose mg U/kg	Number of Doses	Frequency	Test Dose mg/kg	Urinary Citrate: 2 week Pre-treatment mg/24 hrs.	Urinary Citrate: Last 2 Wks. After Treatment mg/24 hrs.	Atypical Tubular Epithelium Developed
A	0.2	13	Every 2 days	5	10-35	20-100	No
B	0.33	13	"	5	10-55	60-160	Yes
C	0.33	13	"	5	10-90	110-290	Not reported
D	0.33	11	"	5	10-75	120-270	Yes
E	0.33	11	"	5	5-40	60-290	Not reported

NOTE: Dog A was not regarded as showing tolerance as judged by the lack of substantial increase in citrate excretion and failure to develop atypical tubular epithelium. The rest of the animals were considered to show tolerance as demonstrated by their survival of the test dose and the increased citrate excretion. In two of the animals atypical tubular epithelium was also found.

Although tolerance can be gained by appropriate doses of uranium the doses must be greater than a certain minimum level which for rats given uranyl nitrate intraperitoneally lies between 0.1 and 0.2 mg/kg. Tolerance once acquired can be lost but may persist for periods as long as six months without additional doses of uranium.[9] On the basis of their own studies, Hodge et al summarised the criteria for recognizing tolerance as:[9] page 1641

1) The ability of an animal to withstand, without typical response, doses of uranium that would otherwise produce this response, e.g. the ability of an animal to withstand ordinarily lethal doses or more without succumbing.

(2) The maintenance of elevated excretion levels of the amino acids for long periods after the termination of a course of uranium injections in the face of a return to normal of other variables and a normal plasma amino acid concentration.

(3) The association of an increased output of citric acid with the characteristic atrophic renal tubule.

That the kidney which has developed tolerance is not a normal kidney and may under certain circumstances be at a disadvantage compared with a "non-tolerant" kidney is shown by the persistence of abnormally high amino acid/creatinine ratios in the urine of rabbits 2 months after the last injection of uranium. Although the ratio was abnormal in each animal at this time, the histological picture was normal. The dosage of uranium used in these experiments was sufficiently high to provoke abnormal renal histology, but regeneration is generally complete in about 3 weeks. Thus it must be concluded that the regenerated kidney in the rabbit is less efficient than the normal kidney in resorbing amino acids.[9] page 1860

From their own attempts to produce uranium tolerance in mice, Tannenbaum concluded, "From a clinical viewpoint, tolerance cannot be considered a practical measure of protection, since tolerance is probably of only limited duration and magnitude and does not prevent accumulation of the poison or chronic damage." Quoted in 12.

The Critical Organ Concentration of Uranium

In the extensive studies of Hodge and his co-workers there is not a very definite statement as to what would constitute a "safe" level of uranium in the kidney. The nearest thing to such a statement being the remark on page 2418 of ref. 9 that "as a guess, 2-3 μgU/g might be deposited without serious tissue damage." A survey of the preceding tables in which the Hodge data is correlated with the measured tissue levels of uranium suggests that this statement is correct, but the avoidance of "serious tissue damage" is not the same as selecting a no-effect level. From the preceding tables the highest tissue level of uranium that is without effect on the morphology of the kidney in dogs and rats is about 1.5 micrograms of Uranium per gram of wet tissue.

Toxicological Studies Other Than Those Associated With The Manhattan Project

Although an outcome of the toxicological studies associated with the Manhattan Project, the 5 year inhalation study of Uranium Dioxide was not reported until 1970. In this study a total of 446 animals were divided into control and UO_2 exposed groups. The exposed group consisted of 110 dogs, 25 monkeys and 200 female rats. These animals inhaled UO_2 dust of approximately 1 micron MMD at a concentration of 5 mgm U/M^3, 6 hours per day, 5 days per week, for periods up to 5 years. After 1 year of exposure the concentration of uranium in the kidney did not exceed 1 µgU/g in rats but after 5 years the level in dogs was about 6 µgU/g and 13 µgU/g in monkeys. The statement is made by the authors "Histologic changes that could be attributed to the inhalation of UO_2 dust were found almost exclusively in the lungs and TLN (thoracic lymph nodes). No description of the kidney morphology is given but a statement is made in the abstract that kidney injury did not occur at the exposure level of 5 mgm U/M^3."[16] It is difficult to reconcile the kidney levels of uranium in this study with the reported lack of injury, although it may be that very early changes in the kidney were not searched for to the same degree as in the earlier studies, as the main interest in the 5 year study was radiation damage to the lungs and thoracic lymph nodes.

Although there are many other studies in the literature that are concerned with various aspects of the effect of uranium on the kidney, no other references have been found which contain information relevant to developing exposure-effect and/or exposure-response relationships in animals. It is understood that such a study has just been concluded but has not yet been published. (P. MORROW, University of Rochester, personal communication.)

In an interim report on this study[46] MORROW et al found that their data in rats and dogs from inhalation studies of UO_2F_2 support the relation between absorbed dose and urinary elimination rate proposed by the ICRP for ^{+6}U compounds. The absorbed dose that was just capable of producing renal injury was about .01 mg kg^{-1} body weight. This the authors note is in contrast to previously reported human and animal studies which suggest 0.1 mg kg^{-1} as an approximate threshold dose for renal injury. The authors conclude in their interim report that their studies suggest that the threshold for damage is about 0.01 mg/kg^{-1} in the previously unexposed subject. The indicators of renal injury were urinary protein, glucose and plasma urea nitrogen. Of these urinary glucose appeared to be the most sensitive early indicator of damage when pre and post exposure values were compared.

SECTION 5

Exposure-Effect and Exposure-Response Information in Humans

For obvious reasons, no single experiment or investigation in which human exposure to Uranium occurs provides all of the necessary data for estimating safe exposure limits. Data derived from humans is of two principal types. Firstly, that in which a known dose of uranium is administered but autopsy data is not available, in which case the administered dose cannot be compared with the histological picture of the kidney or the renal tissue content of uranium, and secondly, that in which there is autopsy data and it may be possible to provide the histological picture and the tissue concentration of uranium but usually the level of exposure to uranium is imperfectly known.

A summary of the relevant studies follows.

Rochester Study

Six hospital patients with good kidney function and with urine free of protein were given injections of Uranyl Nitrate at levels calculated to have no harmful effect. The amount of uranium excreted in the urine was then measured and serial tests of kidney function were carried out. (Tables 18 and 19) Uranium in fecal and blood samples was also measured. Transient proteinuria and catalasuria were noted in the two patients receiving the highest doses of uranyl nitrate >42 µg/kg. Proteinuria was noted in these two patients 4-6 days after injection.[10] From these studies the dose of uranium which produces minimal damage to the kidney tubule in man was determined.

TABLE 18

Patient No.	Sex	Age Yr.	Wt. Kg	Condition	Dose µg/kg	Urinary Excretion Percent 24 h.	Urinary Excretion Percent 5 days
1	m	32	60.5	Arthritis-rheumatoid	6.3	82.3	86
2	f	40	75.5	Cirrhosis of liver	6.3	84.7	87
3	f	24	37.0	Chronic undernutrition	15.8	69.2	71.5
4	m	42	64.0	Chronic alcoholism	30.0	66.6	74.3
5	m	51	65.3	Unresolved pneumonia	42.0	75.3	76.3[a]
6	m	61	55.1	Pulmonary fibrosis	70.9	77.6	87.6
6	m[b]	61	55.1	Gastric ulcer	54.5	57.3	79.5

[a]Collected 4 days only.

[b]A second dose was given to this patient.

PATIENT DATA AND URINARY RESULTS FOR ROCHESTER EXPERIMENT[10]

TABLE 19

Patient No.	1	2	3	4	5	6
Urinary catalase (ml of H_2O)	No change[a]	No change	No change	No change	3.6 (3.7)[b]	9.5 (10.5)[c]
Urinary protein (mg albumin/5 ml urine)	0(0)	0(0)	0(0)	0(20)[d]	0(<5)[e]	
Amino N/Creat.N	No change	0.49 (0.48)	0.26 (0.24)	No change	0.15 (0.11)	0.14 (0.16)
Glom. filtration (ml/min)	-	115 (118)	118 (117)	-	-	99 (93)
Renal plasma flow (ml/min)	-	463 (402)	593 (638)	-	-	482 (499)
Max. tub.excr.cap. (mg/min)	-	70 (79)	-	-	-	95 (83)
Urea clearance (percent normal)	102 (96)	-	43 (52)	-	77 (--)	-

[a]To be read "tests were performed and no change observed." Dash to be read, "test was not performed."

[b]Except as noted average pre-injection values are listed first with average post-injection values in parentheses.

[c]Questionably positive values on days 4 and 5.

[d]Not an average. Listed value occurred on day 4.

[e]Not an average. Traces, <5 mg, noted on days 5 and 6.

SUMMARY OF RENAL FUNCTION TESTS ON ROCHESTER PATIENTS[10]

Boston Study

Eight terminally ill brain tumour patients, 6 of whom were comatose, were given doses of uranium compounds intravenously ranging in amounts from 4 to 50 milligrams of uranium. Excretion of the uranium was then followed by analyzing the urine and blood for uranium.[17] Transient proteinuria and catalasuria were noted in patients receiving more than 70.9 µg/kg uranium. Acute tubular damage was not seen at autopsy. It was estimated that the minimal dose needed to produce catalasuria and albuminuria was about 0.1 mg/kg. The primary justification for this study was an assessment of the possible value of neutron capture therapy. (Tables 20 and 21)

Experiments Using Uranium Injections To Evaluate Skeletal Metabolic Disorders

In a series of studies designed to evaluate the metabolic status of 7 patients with various bone disorders, and 3 control patients without bone disorders, soluble uranium salts were injected intravenously and the rate of excretion measured in the urine over the succeeding 6 days.[10]

Results of Studies

Excretion Data: The 24 hour excretion of uranium as a percentage of the injected dose was very similar in each of the studies and gave a value of 72.1±8.3 (omitting data from 1 of the 24 subjects).

Dose Just Producing Minimal Kidney Injury

Of the 3 sets of data, only that from Rochester and Boston provided information on kidney function and the results of these two studies are in close agreement. The level suggested by the Rochester Study based on a transient rise in urinary catalase and protein levels was 70.9 micrograms of uranium per kilogram of body weight, while similar end points were noted to occur in the Boston patients at a level of 100 micrograms of uranium per kilogram of body weight. Using the data from the Boston patients because tissue levels were available as well as a known interval from the time of injection, it was possible to estimate the biological half life for uranium in both bone and kidney. For bone the half life for uranium calculated from this data is 300 days which agrees with

TABLE 20

Patient	Age Yr	Sex	Wt. kg	Dose[a] µg/kg	Excretion percent dose 24 h	Excretion percent dose Total	Survival post-injection days
1	26	m	55.9	99	59.4	69	2.5
2	47	m	57.4	103	78.0	92	74
3	34	m	60.0	72	83.8	98	566
4	63	f	67.7	165	77.2	85	136
5	39	m	55.9	283	66.5	85	139
6	60	m	56.7[b]	907	49.1	63	18
7	-	-	71.8[b]	573[b]	20.0	68[b]	228[b]
8	-	m	63.2[b]	700[b]	16.9	57[b]	21

[a]These values differ slightly from those listed by LUESSENHOP et al. (1958). We have assumed that the body weights and total dose administered as listed by them are correct. If these numbers are used, the values listed above are obtained.

[b]These data were kindly provided by Dr. S. R. BERNARD.

PATIENT DATA AND URINARY EXCRETION OF INTRAVENOUS URANIUM[10]

TABLE 21

Patient No.	1	6	2	5	3	8[a]
Bone	10.0	4.9	1.4	0.6	1.3	14.4
Kidney	16.6	7.2	0.7	1.2	0.4	1.1
All other tissues and organs	8.4	5.9	1.9	1.2	0.3	15.7
Bone tibia biopsies 0-48 hours average % dose per 7000 g	9.1	6.4	6.5	3.9	4.2	1.3

[a]This patient was injected with uranium tetrachloride.

PERCENT OF INJECTED DOSE PER STANDARD MAN ORGAN OR TISSUE ESTIMATED FROM AUTOPSY RESULTS[10]

that quoted in the National Bureau of Standards Handbook 52 which is based on animal data. Bernard[17] using the Boston data concludes that this biological half life of uranium in the kidney is also 300 days which is at variance with the 15 day figure used by ICRP and NCRP.

There have been few studies in which uranium salts have been given by mouth but in the late 1800's there was some use of uranium as a therapeutic agent for diabetes mellitus. Although doses as high as 925 mg of Uranyl Nitrate 3 times a day were used, no evidence of kidney injury was reported using routine urinalysis. It was found by the treating physicians that larger doses of uranium were necessary to obtain similar results after temporary cessation of treatment.[45]

In 1955 a paper was published in which a description was given of the oral ingestion by a volunteer of one gram of Uranyl Nitrate (0.47 gram U). The subject experienced vomiting, diarrhea and slight albuminuria. From measurements of urinary excretion, it was calculated that the absorption from the gut must have been about one percent. This study, while not shedding much light on the matter of tissue distribution, did lead to a change in the value for the fraction of uranium absorbed from the gut.

In the only other report of oral ingestion with appropriate follow-up measurements, 4 carefully screened hospital patients were given 10.8 mg of Uranium as Uranyl Nitrate. Their urine samples and, in some cases, their feces were collected and analyzed for uranium. With regard to the question of the kidney burden of uranium associated with minimal functional or morphological damage, the oral ingestion studies have thrown little light on it.

Only one report has been found in which intentional inhalation of uranium dust has taken place. The experiment was carried out in an attempt to explain the discrepancy between the levels of dust in some parts of the uranium industry and the lack of disease which those dust levels might be expected to produce.

In this study a single subject was exposed for 17 short periods of, first UO_3 (12 periods) and later UF_4 (5 periods) over an elapsed time of 24 days. Continuous samples of the inhaled dust were taken for particle size determination. The subject's ventilation was recorded and total urinary and fecal collections were made for a period of 32 days and analyzed for uranium. The urinary excretion pattern was biphasic with a rapid excretion phase having a half life of

about 7 hours and the half time for the slow decrease phase being about 4 days. No information on the deposition of uranium in the kidney is available from this experiment and no measurements of renal function were made.[10]

After these intentional exposures of humans to uranium comes a very large body of experience with persons exposed to uranium through their work.

Occupational Exposure

As with many occupational exposures to toxic chemicals, there is a widespread view in the uranium industry that despite the exposure of many hundreds of workers over periods of many years, and remembering that exposure levels have decreased substantially in recent years at many locations, "there has been no great wave of uranium illness."[19] Such beliefs cannot lightly be dismissed but it must be remembered that relatively high levels of prevalence of a disease must often exist before its presence intrudes upon the consciousness of the industry. This is particularly true if the disease is fairly common in the unexposed population, e.g. lung cancer and chronic kidney disease. Therefore, although the belief of those in the industry is important, it is equally important to know how hard the search for ill health has been pressed and the precision of the analytical tools that have been used.

A further point that is often raised in discussing studies of morbidity in industry is that the World Health Organization and International Labour Organization defines health as "not the mere absence of disease." Therefore, it could be argued that to show that uranium is not causing detectable disease in uranium workers is not the end of a concern over the occupational health of uranium workers, but rather is a milestone along the road.

Information Relevant to Determining Critical Organ Concentrations of Uranium

Data from occupational groups that would contribute directly to knowledge about critical organ concentrations and their correlation with disease processes is confined to autopsy material. A number of programmes have been set up to gather such autopsy data but the acquisition of such information is necessarily slow. The published information up to 1971 is shown in the following table.

TABLE 22

ANALYTICAL DATA ON POSTMORTEM TISSUES FROM PERSONS OCCUPATIONALLY EXPOSED TO URANIUM

Case	Exposure			Uranium Concentration (µg U/g wet tissue)				
	Years	Soluble or Insoluble	Degree	Lung	Kidney	Bone	Liver	Lymph Nodes
1	3	insoluble	moderate	0.42	0.071	0.018		
2	5	mixed	low	1.02	0.038	0.048		
3	5	mixed	low	0.06	0.008	0.035		
4	5	mixed	low	0.05	0.03	0.03	0.01	0.03
5	7	mixed	moderate			0.21		
6	9	mixed	moderate	0.05				
7	9	mixed	high	5.4				2.1
8	10	insoluble	moderate	1.7	0.05	0.07	0.01	
9	5	soluble	moderate	0.15	0.08		0.04	
10	3	soluble	low	0.05	0.04			
11	9	mixed	low	0.20	0.04			
12	11	mixed	low	0.02	0.02			
13	3	mixed	low	0.05	0.04		0.02	
14	5	mixed	low	0.05	0.06		0.04	0.12
15	5	not known	not known	0.05	0.06		0.05	0.12
16	3	not known	not known	0.05	0.05		0.02	
17	10	not known	not known	0.06	0.03		0.01	
18	10	not known	not known	0.03	0.02			
19	9	not known	not known	0.12	0.06		0.02	
20	8	not known	not known	0.20	0.04			
21	9	not known	not known	1.6	0.12		0.04	0.32
22	5	not known	not known	0.05	0.05			
23	5	not known	not known	0.05	0.02		0.02	0.10
24	6	not known	not known	0.03	0.02		0.02	
25	10	insoluble	high	1.2	0.14	0.09	0.02	1.8

Adapted from Ref. 10

In addition to this compilation from published data available up to 1971, tissues from 350 autopsies have been analyzed at the Los Alamos Scientific Laboratory. Most of the autopsies were on residents of Los Alamos, New Mexico and the surrounding area with a few cases from other states. This program at Los Alamos was reported on in 1976 and covered the years 1959-71. The following table contains a summary of tissue concentrations in 8 workers potentially exposed to uranium.

TABLE 23

RENAL URANIUM LEVELS
IN WORKERS EXPOSED TO URANIUM

Case	Kidney Tissue Concentration
1	.11 µg/g wet tissue
2	.02 "
3	<MDL* "
4	.03 "
5	<MDL "
6	<MDL "
7	<MDL "
8	.05 "

*MDL = Minimum Detectable Level based on a detection limit of 5 µgU/litre of solution analyzed. For the kidney the MDL was .001 µgU/g.

In the general population 35 out of the 350 cases gave a positive value in at least one tissue (positive = above the MDL); the rest of the tissue uranium levels were below the MDL.

While these tissue levels of uranium are of interest in the light of the animal data previously discussed, they do not allow of any conclusions as to the morphological or functional state of the kidneys as no information on these matters was given in the reports cited. The information may be of value in assessing the hazard of particular exposures when it can be coupled with better exposure-effect data than we now have.

SECTION 6

The Use of Kidney Uranium Levels as a Basis for Setting Limits for Human Exposure to Uranium

To prevent workers suffering from the chemical toxicity of uranium, the tissue concentration of uranium in the critical organ must be kept below the critical concentration. There appears to be little disagreement in the literature that the kidney is the critical organ for chemical toxicity and therefore the concentration of uranium in the kidney is an appropriate way of approaching the setting of limits for human exposure to uranium.

While almost all of the available data points to the kidney as the critical organ, there is one experiment conducted by Hodge et al[9] pages 1253-1258 which, if confirmed, appears to show that the reproductive system is sensitive to a single 24-hour exposure to uranium at the 2% level in the diet, the effect being measurable 9 months after the one-day exposure.

In the extensive program of toxicological studies carried out in connection with the Manhattan Project was an investigation of the effects of uranium on breeding in rats. Two large groups of male and female rats (a control and an experimental group) were maintained on a 2 percent uranyl nitrate hexahydrate diet for a period of one year so that the effects on reproduction could be observed. The experimental diet was provided for 7 months, after which the animals were replaced on the stock ration for the balance of the year.

The results of the breeding experiment showed a serious effect on reproduction: (1) fewer females in the experimental group had litters compared with the control group; (2) the litters in the experimental group were smaller in number; (3) the oestrus cycles were irregular among females in the experimental group.

Because the 2 percent uranyl nitrate hexahydrate diet caused severe interference with nutrition as shown by a marked failure to gain weight at the same rate as the control animals, it was thought that the interference with reproduction might be due to malnutrition. It was therefore decided to repeat the experiment in such a way that the nutrition of the control and experimental animals was maintained at the same level throughout the experiment.

In the second experiment, 50 male and 50 female rats and the same number of litter-mate control animals were selected on the basis of body weight to give groups of almost

identical weight. At weaning the two groups of rats were given a diet containing 2 percent uranyl nitrate hexahydrate for a single 24-hour period. After this single 24-hour period the experimental animals were placed on the same stock diet as the control animals. The growth curves of the experimental and control animals were practically identical for both the male and female animals.

The single exposure of 24 hours to the uranium compound was followed by 252 litters being born to the control female rats through ten months of the study, whereas the experimental rats had 233. This difference of 19 litters represents a decrease of 7% in the number of litters in the experimental group. The number of offspring showed a greater percentage difference between the control and experimental groups. The control female rats had a total of 1958 pups while the experimental female rats had 1725 pups in the same 9 month span. This difference of 233 pups represents a decrease of 12% in the experimental group. The average litter size was 7.9 for the experimental group and 8.6 for the control group over a 7 month period.

While these differences in reproductive performance are not great and may be due to other factors than the single 24-hour exposure to uranium, the increased concern now attached to the effects of chemicals on reproduction, as compared with the time when the Manhattan Project studies were carried out, merits a careful reappraisal of the effects of uranium on reproduction.

With this caveat, the kidney will continue to be taken as the critical organ for the purposes of this report.

Mortality in Uranium Workers

Epidemiology is the study of the distribution and determinants of disease frequency in man.[28] The study of the levels of uranium in the kidney and how these vary in different exposure groups is, therefore, clearly within the realm of epidemiology, although it contains no direct measurement of the state of health of the population involved. This is true because the toxicological investigations have shown that the level of uranium in the kidney is a determinant of kidney damage in animals. As mentioned in the beginning of this report, the equating of a certain level of morphological or functional damage with a "disease" process is a difficult and controversial matter and one which epidemiology is frequently expected to answer. A clear-cut answer cannot, however, be expected unless there is a clear-cut definition of the "disease" whose distribution is being studied.

If death from kidney disease is the definition being used, then it is true that there is no reported evidence linking long-term occupational exposure to uranium to death from kidney disease. This statement is meaningless, however, unless it is known that a search for deaths from kidney disease has been carried out. If such a search has been carried out, it naturally leads to further questions such as: was a control group Used? Were persons leaving the industry followed up to find their cause of death? How valid is a death certificate as a statement of the actual cause of death?

With all of its admitted difficulties, epidemiology is still thought of as providing the only means for keeping a sense of proportion in evaluating the health effects of toxic agents. If, despite minor biochemical changes, workers exposed to certain levels of uranium live as long and suffer from the same diseases both in severity and duration as a properly selected control population, how then are the minor biochemical changes to be viewed?

The epidemiological studies of the mortality of uranium workers have concentrated upon the possible effects of ionising radiation as a cause of death but as death certificates were the source of this information, causes of death unrelated to ionising radiation were also studied. In a survey of a cohort of 18,869 white males employed between 1943 and 1947 at a uranium conversion and enrichment plant in Oak Ridge, Tennessee, workers had been exposed to high levels of uranium dust. These levels are listed in the following table.

TABLE 24

AVERAGE CONCENTRATION OF URANIUM IN AIR BY JOB ACTIVITY AT TENNESSEE EASTMAN CORPORATION IN 1945

Activities	Average Concentration of Uranium in Air
Sublimation (Purification of UCl_4)	300 $\mu g/M^3$
Bottle filling with UCl_4	250 "
Recovery of uranium conversion to UO_3	500 "
Calutron first stage	25 "
UCl_4 production and bottle filling	50 "
Calutron, second stage	25 "

The standardised mortality rate (SMR)(observed number of deaths divided by expected number of deaths based on death rates for U.S. white males) for diseases of the genitourinary system was 0.64 (63 observed, expected 99.04). It is worth noting that applicants for jobs at the Tennessee facility were screened for albumin in the urine and a urea clearance test was carried out as a test of kidney function. If either of these tests were abnormal, the applicant was rejected. Among the workers in the highest uranium exposure areas, the SMR for genitourinary disease was 0.36, (1 observed, expected 2.80). After cautions by the authors on the methodological problems associated with studies of this kind, they conclude "short-term follow-up studies in the 1940s and 1950s of uranium workers exposed for several months or years to high levels of soluble uranium compounds (i.e. UF_6) showed only transient kidney damage (proteinuria) and no evidence of permanent effects. The present findings on mortality (to 1974) from diseases of the genitourinary system are in agreement with this conclusion, but data on morbidity are lacking for the present group."[29] Only one other study of mortality in uranium workers has been found in which all causes of death were reported upon. In this study mortality from all causes for 104 men who had worked in six uranium mills between 1950 through 1967 was recorded. The 104 deaths were slightly under the 105.11 deaths that would have been expected if the death rate for the white male population of the area had prevailed. Apart from the excess deaths due to malignant disease of the "lymphatic and hematopoietic tissue other than leukemia," no other observed vs. expected mortality groupings showed significant differences.[30] No epidemiological studies of morbidity among uranium workers have been found.

SECTION 7

SETTING LIMITS FOR HUMAN EXPOSURE TO AIRBORNE URANIUM DUST IN INDUSTRY

The need for a standard for airborne uranium arose with the development of the Manhattan Project in the U.S.A. The medical department of the Manhattan Project in 1944 issued an interim guide for the maximum permissible concentration of uranium in air of 150 micrograms/M^3. This figure was picked by analogy between the known nephrotoxic action of lead and uranium and the fact that the limit for lead was achievable in industry and, therefore, represented an attainable goal for uranium. The tremendous toxicological studies at the University of Rochester gave rise to the following interpretations of the data produced by persons involved in the studies. Stokinger, in summarizing the uranium inhalation studies, states:

> "One of the chief purposes of the chronic-exposure studies was to obtain information sufficient to allow the establishment of maximal allowable concentrations (MAC) of, if possible, safe concentrations of uranium dust for prolonged exposure. The levels were therefore set sufficiently low to incur either (1) no injury or (2) just minimal response. Safe levels of exposure could be set from (1) and the factor of safety could be set from (2). Based on the chronic-exposure studies using insoluble dusts the MAC selected was 10 mg U/M^3 but for soluble dusts it was felt the levels must be lowered to 50 micrograms of uranium per cubic metre of air, (UF_6UCI_4 or Uranyl nitrate hexahydrate) before exposure approaches a non-injurious level." (9) P1659.

This level is restated on page 2241 of Ref. 9: "The air concentration of 50 micrograms of uranium per cubic metre as soluble uranium dusts is recommended as a tentative M.A.C." The figure of 10 mg was based solely on chemical toxicity considerations and when radiation was considered as the limiting factor for insoluble uranium dusts, the M.A.C. was set at 100 micrograms of uranium per cubic metre.

Hursh (Ref. 9, page 245) has pointed out an apparent contradiction in the published M.A.C. from the Rochester workers in the AECD report 2784 in which, after recommending 100 µg/U/M^3 for insoluble uranium, the following statement appears: "From the standpoint of chemical toxicity, the suggested M.A.C. range of 200 - 250 µg/M^3 allows a reasonable margin of safety." Thus, based on the same data, the same investigations put forward two different M.A.C. values for insoluble uranium dusts.

In 1947, K. Z. Morgan calculated an M.A.C. based on the lung as the critical organ and that 25% of the amount of uranium inhaled was retained in the lung for an effective half life of 60 days. The figures that Morgan arrived at were 210 µgU/M^3 for continuous exposure and 613 µgU/M^3 for occupational exposure.[40] Morgan went on to suggest reducing these figures by a factor of ten because of the non-uniform distribution within the lung.

At a conference in September of 1949, the concept of the reference man was set down and a lung model was agreed upon. As Hursh says in Ref. 12, page 27: "These were the necessary tools for the biomathematical approach which was to take over the estimation of limits for radioactive nuclides. The role of the biologist became that of supplying constants for the precise mathematical formulations." The exposure limit arrived at by the Chalk River conference was a single value for both soluble and insoluble uranium of 146 micrograms/M^3 and was based on chemical toxicity to the kidney.[12 Page 28] Hursh, in reviewing the documentation at the time of the Chalk River conference and subsequent correspondence, comments: "It is clear that at Chalk River, the conference decided to raise the RBE for alpha particles from 10 to 20. It is probable, though less clear, that they decided to recommend 25 µg/M^3 as the MPCa for uranium, both soluble and insoluble."[10 page 247]

At a meeting in Chalk River, an international meeting of health physicists also meeting in September 1944 endorsed the Rochester proposal and accepted 50 µgU/M^3 for a 40 hour week occupational exposure. Bernard has pointed out that a careful reading of the reports of the Rochester animal exposure data shows that the average duration of exposure for all animals in the inhalation studies was 28 hours and not the 40 hours assumed for human exposure limits. Thus, if the Rochester workers suggested 50 µgU/M^3 based on an actual exposure of 28 hours, the exposure limit for 40 hours should be proportionably reduced to a figure of 35 µgU/M^3.

The gathering confusion over the permissible limits on exposure for uranium was further compounded by a recommendation of the International Commission on Radiological Protection in 1954 that 90 $\mu gU/M^3$ be accepted as a continuous exposure limit for both soluble and insoluble uranium. This was later amended by the same organization to 210 $\mu gU/M^3$ for soluble uranium and 180 $\mu gU/M^3$ for insoluble, both figures being for a forty hour work week. In 1957, the American Conference of Governmental Industrial Hygienists (ACGIH) recommended 50 and 250 $\mu g/M^3$ respectively as the Threshold Limit Value (time weighted average exposure for an 8 hour day 40 hour week). In 1968, the ACGIH recommended that the Threshold Limit Value for both soluble and insoluble uranium be set at 200 micrograms uranium per cubic metre. The reason for the change was that "in a report of the health experience in one plant after 15 years (1958) in which exposure was to natural uranium and its compounds, but not UF_6, no abnormal clinical findings were traced to uranium as a cause. Similar experience was reported at other uranium processing plants to 1958. In the period from 1958 to 1967, during which time the experience was extended to a maximum of 25 years, no further evidence of uranium effects in these workers has come to light."[41] This action of the ACGIH raises two important points. The first relates to the setting of standards based on data that only the Threshold Limit Value Committee has seen and is not in the open literature, and the second is the reason for raising a level that appeared to be safe. As no beneficial biological action of uranium in humans has been reported, raising of exposure limits must be done for reasons other than health protection. These reasons may have validity but a number of people would probably argue that the criteria for arriving at a judgment based on socio-economic considerations should be set down along with those for making judgments of exposure response and exposure effect relationships. On a more technical note; while it is reassuring that no ill effects have been found in the uranium workers mentioned in the TLV documentation over a period of 25 years, details of the health data obtained have not, as far as is known, been published so that a judgment can be made as to its adequacy, and it is not known whether an attempt was made to follow up workers who left uranium exposure. By analogy with persons occupationally exposed to lead, only comparisons between the results of appropriate tests in exposed and matched control subjects would be likely to reveal subclinical kidney disease.[42]

The ACGIH Threshold Limit Values in their entirety or a selected list of the values are used in many industrialized countries including Canada, U.K., U.S.A. and Sweden. In the U.S.S.R., exposure limits for workers, as published, are often lower than those used in the West by a factor of 5 to 10. These differences, where they occur, appear to be due to a difference in philosophy. Whereas in the U.K., Canada and the U.S., the exposure limits are set as high as possible without causing harm to more than a few people, the Soviet philosophy is that "exposure to a harmful substance should not cause any somatic, psychic, intellectual or emotional after-effects later on in a person's life, or prevent that person from functioning normally in a social environment."[43] Effects are considered harmless when the changes induced do not exceed the limits of statistical standards or cause weakening of compensatory, adaptive, defence and reparative mechanisms. As the documentation for the exposure limits used in the U.S.S.R. are seldom available in the West, it is difficult to discuss them in detail and in particular. No supporting documents were available for derivation of MAC's for uranium in the U.S.S.R. Such limits may be more stringent than present technological capabilities of pollution control, but are useful as goals to be attained by future technology.[47] This view is at variance with the official view in the U.S.S.R.

"In the U.S.S.R. an official permanent commission regularly engaged in determining the MAC in the air of the workroom area, has assigned such values for the Ministry of Health since 1956.

"With the advice of the Institute of Industrial Hygiene and Occupational Diseases of Moscow and the U.S.S.R. Academy of Medical Science, these values are declared as Soviet standards. They are regarded as legally binding as absolute values."[1]

In both human and animal studies, the lowest concentration of the toxic agent which produces an "effect" when compared with a control group is considered to be the effective concentration for that substance.

In the case of standards based on sensory or behavioural changes, the threshold reached by the most sensitive person is accepted as the basis for determining MAC's. It need not be perceived by all subjects.[49]

The concept of accepting the first change in an animal's normal behaviour or physiology as the effect level for that substance poses some problems as some of these changes may be evidence of adaptation or even mere perception of the presence of the test substance. For these and other reasons, the standard setting process used in Eastern Bloc countries has not been adopted in the West although, in general, there has been a tendency for western exposure limits for chemicals in the workplace environment to be reduced with time. One of the difficulties in accepting, or even discussing, the standards promulgated in Eastern Bloc countries is the lack of published supporting data showing the derivation of the standard. The publication of such supporting data is now considered essential by most standard setting bodies in western countries. A further hindrance to the acceptance of the U.S.S.R. standards is the view expressed by many visiting occupational hygienists that these MAC values represented ideal objectives and at the worksites more "practical" exposure limits were used.[48]

TABLE 25

OCCUPATIONAL EXPOSURE LIMITS FOR AIRBORNE TOXIC SUBSTANCES

I.L.O. GENEVA, 1980[1]

URANIUM

Country	Year	Compound	Limit (mg/m^3)
Australia	1973	Insoluble	0.20
		Soluble	0.20
Belgium	1974	Insoluble	-
		Soluble	0.20
Bulgaria	1971	Insoluble	0.075
		Soluble	0.015
Czechoslovakia	1971	Insoluble	0.075
		Soluble	0.015
Finland	1975	Insoluble	-
		Soluble	0.20
Germany (GDR)	?	Insoluble	0.075
		Soluble	0.015
Germany (FDR)	1976	Insoluble	0.25
		Soluble	0.25
Italy	?	Insoluble	0.20
		Soluble	0.20
Netherlands	1973	Insoluble	-
		Soluble	0.20
Poland	1976	Insoluble	0.075
		Soluble	0.015

(Continued)

TABLE 25 (Continued)

OCCUPATIONAL EXPOSURE LIMITS FOR AIRBORNE TOXIC SUBSTANCES

I.L.O. GENEVA, 1980[1]

URANIUM

Country	Year	Compound	Limit (mg/m^3)
Switzerland	1976	Insoluble Soluble	0.25 0.05
U.S.S.R.	1971	Insoluble Soluble	0.075 0.015
U.S.A.			
NIOSH	1978	Insoluble Soluble	0.25 0.05
ACGIH	1978	Insoluble Soluble	0.20 0.20
NCRP	1978	Insoluble Soluble	0.180 0.210
NRC	1978	Soluble Insoluble	0.200 0.147
Yugoslavia	1971	Insoluble Soluble	0.25 0.05
Council of Europe	?		0.25
ICRP	1978	Insoluble Soluble	0.180 0.210

The maximum allowable concentrations of uranium in the workplace vary from country to country.

The Eastern Bloc countries have generally lower MAC levels than western European or North American countries. Most countries with an exposure limit for uranium follow either the U.S.A. or the U.S.S.R. practice, and as the U.S.S.R. does not publish the criteria upon which their values are based, it is not possible to compare the scientific validity of the two standards. The official limits set for airborne occupational exposure in various countries and by certain standard setting bodies are given in table 25. The discussion of the validity of the exposure limits based on the United States data will be found in Section 4.

In looking at the data available for setting exposure limits for uranium, there is a clear dilemma. On the one hand, there are very good animal studies, the very size and thoroughness of which may have had the effect of stifling further research and, on the other hand, there is a consensus among those connected with the industry that a large number of workers have been exposed to uranium over a period of nearly forty years at levels above those suggested by the animal studies and no demonstrable damage to health has taken place as a result of those exposures to uranium.

Writing in 1975 about the lack of reported human health effects, Eisenbud said: "This statement could be made with more confidence if there had been more thorough follow-up of those who worked in the early production plants . . . . The invitation to prepare a paper for this meeting came to me about 20 years after we first summarized the field experience in 1955. No additional cases have been added to the series that I prepared at that time. It is even more disappointing to find no reports of systematic follow-up of the hundreds of employees who were so heavily exposed during these years."[44]

In the face of good data suggesting a lower limit of exposure based on animal work and very limited documented information on the health effects of long-term human exposure, it is probable that the lower limit will prevail if the present levels are challenged. To help resolve the different values suggested by these two types of experience, well planned epidemiological studies would be necessary.

SECTION 8 BIOLOGICAL MONITORING

Urine Analysis for Uranium

The use of urine as a biological monitoring medium has always been attractive since adequate analytical techniques became available, but it is probably fair to say that the interpretation of the results has proven frustrating. For a biological monitoring program to be useful, it must have clearly defined goals and the reasoning behind the program must be understood by those involved. If these elements of the program are lacking, there is a great possibility that one or other of the parties involved in the program will misinterpret the data or have unrealistic expectations of the results. The problems associated with urine uranium levels have been debated on several occasions at various symposia[18,12,31] and as the situation has not changed appreciably over the years, the headings used in Ref. 31 will be used here as a resumé of the problems.

Objectives for a Urine Sampling Program

1. To detect brief exposures to personnel that go undetected by air sampling or other evidence;

2. To monitor exposure levels in conjunction with or in place of air sampling;

3. To determine "body burden."

Discussion of Objectives

1. Within 24 hours of the entry of uranium into extracellular fluid, half to three-quarters is excreted into the urine. Thus, analysis of urine for uranium provides a ready check on the occurrence of recent internal contamination, but only in individuals who do not have an appreciable pre-existing body content is it possible to relate urinary levels to body or organ content.[32] Because of the rapid excretion of that uranium which enters the extracellular fluid compartment, Lippmann has stated "The collection of urine samples to detect brief exposures not detected by air sampling is usually a waste of time and effort."[31] One of the problems frequently pointed out in discussions of the value of urinalysis to detect a brief exposure is the great dependence that must be placed upon a single urine analysis

because by the time the first sample has been analysed, a second sample taken in an attempt to confirm the initial high reading may reveal normal or only slightly elevated values. The question then arises, was the first reading correct, the result of contamination[33] or analytical error? Of course, collateral information may support or refute the occurrence of a brief abnormally high exposure in which case the urine analysis may serve as a trigger to search for other data, but some feel that urine sampling of sufficient men with sufficient frequency to detect a brief exposure is a very inefficient way of spending money.

2. It has long been an axiom among some occupational health professionals that the worker is the best sampler of the workplace environment and that a biological monitoring program by measuring a part of the material actually absorbed is the best measurement of the level of environmental contamination. These arguments are only as valid as the assumptions upon which they are based. The most important assumption in the above case is that the concentration of uranium in the urine is directly related to the level of absorption. This relationship can be quite complex. For instance, a Monday morning urine value of 25 µg/litre, could result from any one of the following combinations:

   (1) A steady state contribution of 25 micrograms/litre;

   (2) A steady state contribution of 15 µg/litre plus 10 µg/litre from a Friday exposure of 100 $\mu g/M^3$;

   (3) A steady state contribution of 5 µg/litre plus 20 µg/litre resulting from a Friday exposure of 200 $\mu g/M^3$.

   If the analytical problems associated with basing a reading on a spot sample of urine are then added, the confusion is twice confounded.

3. Under experimental conditions urine samples can provide good information upon which to base estimates of the body burden of uranium, but in a working situation the number of variables, several of which have already been mentioned, make an estimation of body burden based on urinalysis results very risky. To which can be added the very considerable individual variation demonstrated under even ideal conditions. For example, Hursh made

an analysis of the excretion data on the six hospital patients at the University of Rochester previously referred to. In the table that follows, are listed the dose of uranium known to have been injected, the estimated dose based on analytical data derived from several 24-hour urine collections and the range of individual estimates.

TABLE 26

AN ESTIMATE OF INDIVIDUAL BIOLOGICAL VARIATION[34]

Patient No.	True Dose µgU	Average Estimated Dose µgU	No. of 24-hour Urine Samples	Range of Single Estimated
1	385	310	3	185 - 431
2	476	223	3	170 - 292
3	584	482	6	275 - 657
4	1,918	2,143	7	1,690 - 2,920
5	2,746	3,047	3	1,842 - 4,560
6	3,910	14,286	6	7,840 19,400

Urinalysis is probably most reliable and therefore some would say most useful when the data from a number of persons having comparable exposures are pooled. The correlation between urine levels of uranium and variations in the air levels of uranium for a given process area may then be quite good. An example of this type of data is shown in the following table.

TABLE 27

EXPECTED AND ACTUAL URINE URANIUM LEVELS[35]

FOR JOBS IN THE 25 TO 75 µg/M^3 EXPOSURE RANGE

No. of Jobs	Weighted Air Dust Exposure Range	Weighted Air Dust Exposure Average	No. of Urine Samples	Average Urine Concentration µg/ℓ Found	Average Urine Concentration µg/ℓ Expected*
4	26-31	28	42	0.023	0.017
4	35-49	38	47	.026	.023
4	58-64	63	52	.026	.038
5	65-75	70	63	.042	.042

*Calculated values assuming 50 µg U/M^3 ≡ 0.030 µg litre in urine.

Another important method of using urinalysis data is to plot the frequency of urine uranium levels above some arbitrary level suggested from previous experience as indicative of the need for some particular type of action. A rise in the frequency of abnormal urine levels is a useful guide to deteriorating industrial hygiene.

Despite a number of meetings largely or entirely devoted to the subject of the value of urine testing in uranium workers, there appears to be no consensus as to its usefulness. The situation appears to be in the same state that prompted Neuman to say in 1958:

> " . . . the analysis of urine for uranium - does not tell us the whole story. I don't think we should be surprised by this. It is a poor measure of any kind of exposure, of air concentrations, of lung level, bone level, or any level. But it is one of the most convenient and one of the best we have; therefore, its shortcomings have been dwelt on."

Analytical Methods for Uranium in Urine

All measurement techniques involve uncertainties arising from many sources and although these uncertainties can usually be gradually reduced, they can never be entirely eliminated. The process of reducing the uncertainties or errors depends upon identifying and accurately measuring the individual sources and level of error.

Because a degree of uncertainties (error) is inherent in the process of taking samples and analysing them, it is essential in making a judgment as to the quality of the results of such a process that the magnitude of the errors should be known. There are two main classes of error, the determinate ones which contribute constant error or bias, and the indeterminate ones which produce random fluctuations in the data. Both classes of error can be detected and measured by using tests of accuracy and precision. Accuracy describes the relationship between the amount of an element or compound accounted for in the analytical procedure to the amount actually present. Precision is a measure of the analytical method's variability when repeatedly applied to a homogenous sample under controlled conditions.[36]

Uranium may be measured by three methods: mass spectrometry, a fluorimetric method or radiometric methods.

Fluorimetric Analysis

The basic principle is that sodium fluoride fuses with a small amount of uranium, and when exposed to ultraviolet light, emits fluorescence. The fluorometric method is best suited for the analysis of relatively pure substances, as organic material may cause inhibition of the uranium fluorescence.[10] With this method 0.5 ng of uranium in a biological sample can be measured with an error of ± 5 percent by determining the intensity of emitted light.[37]

Radiometric Analysis

Alpha counting is the radiometric method of detecting uranium based on the spontaneous radiation emitted by uranium and its decay products.[38] Both air and biological samples can be analyzed by the alpha counting method.

The counting efficiency can vary widely, depending on the counter and type of sampling medium used to collect the sample. The counting efficiency may be determined by fluorometrically analyzing samples and comparing the results with the alpha count. As a routine procedure, standard samples are counted to assure that the counter is working it properly.

Problems of Analysis

Measurement of uranium in urine is often used to assess the occupational hazards from uranium dust to workers in the uranium industry. In Canada, most bio-assay programs are carried out in-house by the uranium companies, although some measurements are made at government and private consulting laboratories. The Radiation Protection Bureau evaluated the accuracy of ten laboratories for determining urinary uranium levels. The Bureau sent a spiked sample containing 5-45 micrograms uranium per litre to ten laboratories for analysis. The results are presented in Table 28.[39]

TABLE 28

Results of 1978 Uranium in Urine
Interlaboratory Comparison (µgU/l.)

	LABORATORY										
SAMPLE	L	M	N	O	P	Q	R	S	T	U	SPIKE
A	16	20	18	20	26	<10[a]	18	16	19	12	20
B	35	41	89	43	44	38	47	40	47	36	45
C	23	23	36	26	23	13	19	19	21	17	20
D	13	7	35	20	8	20	6	7	6	6	5

[a]Considered as 5 for purpose of calculations

As some laboratories did not include standard deviations with the results, the laboratories were assessed on the basis of mean values using the variance index as described by Whitehead.[40] Laboratory variance index sources were calculated by summing the variance indices for each sample. Indices less than 50 were scored as zero and the mean variance score calculated (Table 29). The laboratories were then assessed as excellent, good, experiencing some difficulty, and experiencing major difficulties, according to the criteria shown in Table 30.

TABLE 29

Variance Indices for the Analysis of Uranium in Urine

	LABORATORY									
SAMPLE	L	M	N	O	P	Q	R	S	T	U
A	67	0	33	0	100	250	33	67	17	133
B	63	25	275	13	6	44	13	31	13	56
C	50	50	267	100	50	117	17	17	17	50
D	80	20	300	150	30	150	10	20	10	10
Variance Index Score	260	50	842	250	150	517	0	67	0	239
Mean Variance Index Score	65	13	211	63	38	129	0	17	0	60

TABLE 30

Evaluation of Laboratories for the Measurement of Natural Uranium in Urine

Evaluation	Modified Variance Score	Laboratories
Excellent	<25	M,R,S,T
Good	25 - 50	P
Experiencing Some Difficulty	50 - 100	L,O,U
Experiencing Major Difficulties	>100	N,Q

The fact that some laboratories had high scores in this study was taken by the authors as an indication that a quality control program of this type is needed in Canada.

This short paper has been quoted rather fully because a search of the literature has failed to show another similar reference in which the results of urinalysis for uranium have been critically examined. Considering the widespread use of urinalysis in the biological monitoring of uranium workers, this lack of published critical evaluations raises questions about the general level of laboratory performance which cannot be answered at this time. It is quite possible that laboratories carrying out urinalysis for uranium are also carrying out their own, satisfactory, quality control programs but it is noted that in Canada,at least at the time of the intercomparison program described in reference 39 in 1980, there was no mention of any such private program and the concept of a government quality control program was welcomed.

Urinary Protein

There is normally a small amount of protein present in the urine of human adults. The amount varies from 30-150 µg daily.[50] The amount of protein may be increased under physiological conditions such as intense physical exertion, pregnancy, after taking a protein-rich diet and in cases of fever. The normal renal glomerulus behaves as though it has pores of 100Å in diameter and may allow the passage of molecules as large as 70,000 daltons, (1.16×10^{-19} g) depending on the molecular shape. Such molecules just include plasma albumin but exclude most plasma globulins. Albumin appearing in the glomerular filtrate may be absorbed in the proximal renal tubule; therefore, albumin in the urine may increase if there is glomerular damage or failure of absorption in the proximal tubule. As noted earlier in this report, uranium is known to damage both the glomerulus and the proximal convoluted tubule of the kidney. The proteinuria which may result from uranium exposure is one of the earliest signs of poisoning but proteinuria itself is non-specific and can be caused by a variety of toxic agents.

There are few published reports of the frequency of proteinuria in a population occupationally exposed to uranium and none which would allow an exposure-response relationship to be described. In a report based on regular medical examinations of workmen employed in uranium reduction plants and in uranium calcining plants in the Republic of South Africa, C. C. FREED stated that "up to the present time there has been no evidence of any injury or disease which can be attributed to the minimal exposure to uranium encountered in the plants. Pathological proteinuria has been rare; we have not been able to correlate such proteinuria with high levels of uranium excretion. Other workers report the appearance of proteinuria in association with a uranium concentration above 0.1 µg/CM^3." "In a total of 10,598 examinations, proteinuria was recorded on 243 occasions. In every case, urine was re-examined, and in the great majority subsequent examination revealed the absence of protein and in only a very few cases was proteinuria a persistent feature. In these cases other medical causes were found responsible."[51]

TABLE 31

Year	Occupational Group**	Samples Examined	Protein* 2-5	Average Uranium Conc. µg/cc
1959	I	508	8 (1.5%)	0.010
	II	1,208	30 (2.4%)	0.005
1960	I	766	14 (1.8%)	0.010
	II	1,041	16 (1.5%)	0.005
1961	I	223	1 (0.4%)	0.011
	II	1,052	15 (1.9%)	0.006
1962	I	244	3 (1.2%)	0.012
	II	848	25 (2.9%)	0.006

URINE SAMPLES EXAMINED 1959-62

* Protein 1 = Nil 2 = Trace 3 = + 4 = ++ 5 = +++

** Group I Those regularly exposed to uranium
Group II Repair and maintenance workers

Although this paper provides only sketchy data, it is quoted because it is the only paper that was found that discussed the occurrence of proteinuria in uranium workers.

B_2 Microglobulin

B_2 microglobulin is a plasma protein having an approximate density of 11,800 daltons (1.96×10^{-20} g/cc). It is a part of the histocompatibility antigens on cell membranes and is freed into body fluids after dissociation from them during their normal turnover. It is released at a constant rate in normal subjects and readily filters through the glomerular capillary wall, over 99.9% being reabsorbed and catabolised in the proximal tubule.[57]

Renal tubule disorders are characterised by the excretion of low molecular weight plasma proteins including B_2 microglobulins (B_2M) whereas the excretion of albumin is more characteristic of glomerular disease. The estimation of B_2 microglobulin has not been reported in uranium workers but it has been used to measure kidney damage in cadmium workers.[52] Cadmium causes tubular proteinuria[53] and in this respect is similar to uranium in its toxic effects.

The B_2M concentrations were estimated on "spot" samples of urine and the values adjusted to a urine specific gravity of 1016, the average urine concentration found for this particular workforce. A raised B_2M was defined as a reading in excess of the upper 97.7 percentile of the pre-employment results, i.e. prior to entering cadmium exposure. In this study five known cases of cadmium-induced proteinuria where exposure ceased up to 15 years previously had raised urinary B_2M concentrations. Moderately raised concentrations were found in seven other men with a history of cadmium oxide exposure and in whom proteinuria had never been detected.[54] It has not yet been shown that a raised B_2M alone is correlated with increased morbidity and/or mortality rates. Also if an elevated B_2M value is associated with permanent kidney damage, its value as an "early warning system" in a preventive medical programme may be limited. B_2M measurements have been used in epidemiological studies as a criterion of an adverse effect of cadmium[54] and they would appear to be of potential benefit in the experimental medical surveillance of uranium workers.

Urinary Enzymes

The presence of enzymes in the urine is a sensitive index of minor tubular lesions and precedes the finding of abnormal renal function tests. Enzymes gain access to the urine either by impaired glomerular filtration, or as a result of desquamation of kidney tubular cells. With intact kidney functions, the excretion of enzymes is confined to those with MG values less than about 60,000.[50] In experimental uranium toxicity in animals, raised alkaline phosphatase and lactic dehydrogenase (LDH) activity has been found, while in uranium mining workers 30% had a significant rise in urinary alkaline phosphatase.[50] In experimental studies in animals LDH appeared to be the most sensitive indicator of kidney damage[55] and it is an appropriate enzyme to measure in uranium exposure, as a raised LDH level in the urine indicates a cellular lesion leading to an increased permeability of the cell membrane as occurs with uranium toxicity. One obvious

problem in using both proteinuria and enzymuria as an index of uranium toxicity is the phenomenon of tolerance discussed earlier. While tolerance is not known to take place in man, the evidence from the animal studies would strongly suggest that the same phenomenon is likely to occur in man.

If "tolerance" does take place with chronic exposure to uranium, it presents two problems in designing programmes for the protection of the health of uranium workers. The first of these is the detection of tolerance, since once it has developed the clinical tests of renal function may be normal. The second problem is that of interpretation. Is tolerance to be viewed as a harmful effect?

With regard to the problem of detection, this could be overcome if a suitable battery of tests, or even a sufficiently sensitive single test were given prior to occupational exposure to uranium and thereafter at regular intervals. If the intervals are properly selected, it may be possible to detect the acquisition of "tolerance" as previously normal tests of renal function become abnormal and later revert again to normal. The underlying assumption behind this interpretation of the results of a series of test results is that the exposure to uranium remained fairly constant during the period of the reversion phenomenon. This assumption could be tested by reviewing the available uranium exposure data.

The question of how to interpret the occurrence of the "tolerance phenomenon," should it be detected, is not easy because it depends upon the philosophy adopted by the standard setting agency.

As has been discussed earlier, there is evidence from animal studies that the kidney that becomes tolerant to uranium is not a "normal" kidney. If it is assumed that any change in function after occupational exposure is not acceptable, then the phenomenon of tolerance would indicate that the exposure is too high. To try and clarify this situation, it is important to collect prospective data to establish whether minimal abnormalities of urinary function which later revert to normal are associated with any long-term increase in the risk to the health of the exposed individuals.

Concentrating Ability of Kidney

Urine concentrating ability is among the more sensitive tests of renal damage used in animals.[56] The ability to produce a more concentrated urine indirectly measures the capacity for active reabsorption of inorganic ions and water by tubular cells. Although concentrating ability has been shown to be a sensitive test of renal function in animals under carefully controlled conditions, it is much more difficult to apply to the much less well controlled environment of the workplace. It must also be recognized that a decrease in the ability of the kidney to concentrate urine is a non-specific effect produced by many factors other than uranium, one of the most important of these factors being age. Performed at regular intervals, a measurement of the specific gravity of the first morning test of urine is a simple and potentially valuable test if longitudinal values in the same individual are studied and also when average values for groups having different exposure levels to uranium are compared.

The Value of Urinalysis as a Means of Biological Monitoring in Uranium Workers

A. Urine Uranium Levels

As has been previously discussed, "spot" urine samples give little information on the critical organ concentration of uranium and are therefore of little value in detecting acute overexposure situations in individuals, but a well-planned program of urinalysis can have a limited but useful value as an index of the exposure of groups of workers to uranium. It must be conceded that biological monitoring is often introduced when the environmental levels of a substance leave a relatively small safety margin. In such circumstances, biological monitoring may provide additional information indicating errors in the environmental measurements and/or additional sources or routes of exposure.

Biological monitoring should, however, never be considered a substitute for the strict control of environmental contamination, and such programmes of biological monitoring may be criticised as appearing to place the emphasis for health protection upon administrative measures directed at the worker rather than engineering measures directed at the working environment. With these important caveats in mind and in the knowledge that biological monitoring programmes have been in place at many sites in the uranium industry for many years, they are probably of value in showing the general trends of environmental contamination.[50]

Where biological monitoring programmes have been conducted for many years, the withdrawal of such a programme would probably be interpreted as a reduction in the level of health surveillance and therefore of health protection.

For other urinary constituents, biological monitoring programmes must be considered experimental but in situations where uranium exposures remain close to the present allowable limits, consideration should be given to instituting carefully planned studies of enzyme levels, protein, β_2M, urinary concentration and citrate excretion to help establish whether the present uranium exposure limits as they relate to chemical damage to the kidney are adequate to protect the long-term health of uranium workers.

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